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Brigadier General Daniel F. Perugini

AMEDD Center and School Welcomes BG Perugini

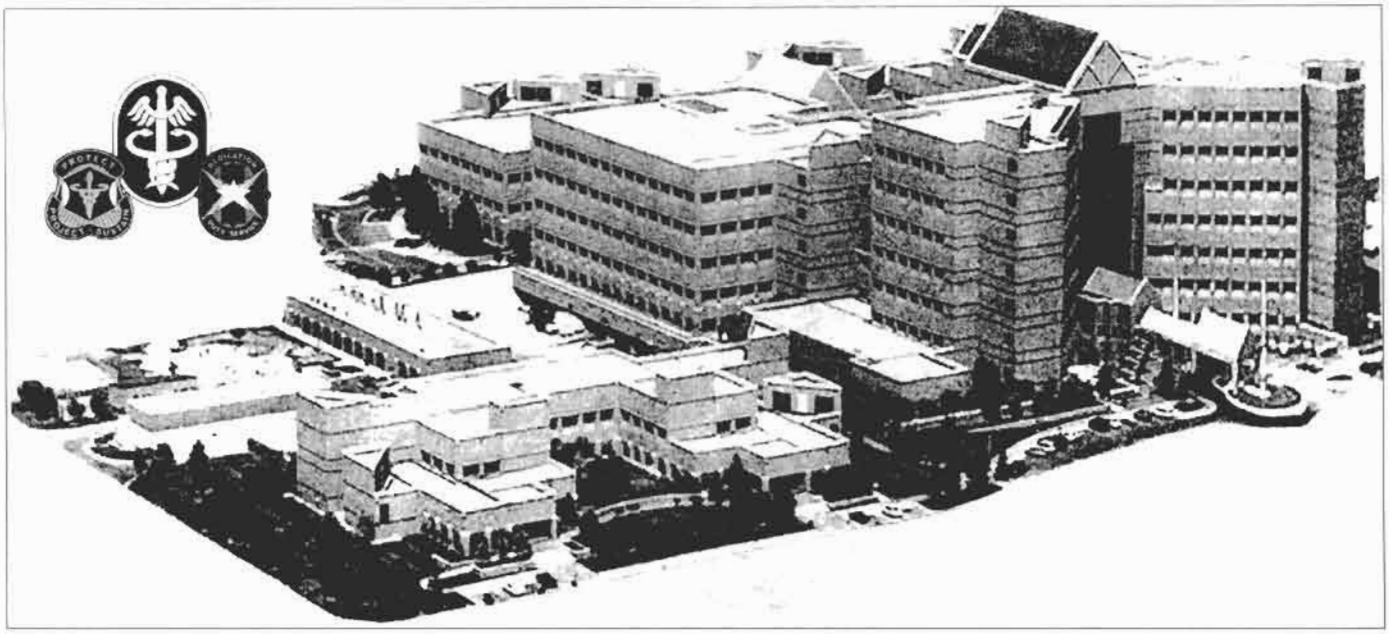
Brigadier General Daniel F. Perugini is the new Commander, U. S. Army Medical Department Center and School and Fort Sam Houston, succeeding MG Darrel R. Porr. He comes to the Home of Army Medicine from his position as Commander, Brooke Army Medical Center (BAMC) and Great Plains Regional Medical Command, Fort Sam Houston, TX.

Brigadier General Perugini entered military service in 1973, following his graduation from the University of Health Sciences College of Osteopathic Medicine. He completed his internship at BAMC in 1974 and his Family Practice Residency at DeWitt Army Community Hospital, Fort Belvoir, VA, in 1976. He is a graduate of the U.S. Army Command and General Staff College and the Industrial War College. He is also a Diplomate of the American Board of Family Practice, the American Osteopathic Board of Family Practice, and a Fellow of the American Board of Family Physicians.

Previous key assignments include Division Surgeon, 8th Infantry Division (Mechanized), Federal Republic of Germany; Commander, Medical Element and Command Surgeon, Joint Task Force Bravo, Palmerola, Honduras; Ambulatory Care/Family Practice Consultant, Office of the Surgeon General, Washington, DC; Commander, Winn Army Community Hospital, Fort Stewart, GA; Deputy Commander for Clinical Services, Eisenhower Army Medical Center, Fort Gordon, GA; Commander, 18th Medical Command and 121st General Hospital and Surgeon, Eighth U.S. Army and U.S. Forces, Korea; and Commander, Womack Army Medical Center, Fort Bragg, NC.

Brigadier General Perugini's military awards and decorations include the Legion of Merit (3 OLC); Defense Meritorious Service Medal; Meritorious Service Medal (3 OLC); Army Commendation Medal (1 OLC); Army Achievement Medal; Physician Recognition Award of the U.S. Surgeon General; Order of Military Medical Merit; Order of National Security Merit Samil Medal-Republic of Korea; and TRICARE Management Activity Executive Director's Award 2000.

Brigadier General Perugini is married to the former Coleta C. VanDeLaar. They have a son, 1LT Daniel Perugini, U.S. Army, and a daughter, Angela Nicole.



U.S. Army Institute of Surgical Research

From the USAISR Commander

COL John B. Holcomb

The U.S. Army Institute of Surgical Research (USAISR) is part of the U.S. Army Medical Research and Materiel Command (USAMRMC) and is collocated with and under operational control of Brooke Army Medical Center (BAMC). The USAISR is dedicated to both laboratory and clinical trauma research. Its mission is to provide requirements-driven combat casualty care medical solutions and products for injured soldiers from self-aid through definitive care across the full spectrum of military operations; provide state-of-the-art trauma, burn, and critical care to Department of Defense beneficiaries around the world; and provide Burn Special Medical Augmentation Response Teams.

The Institute's Burn Center admits approximately 300 patients annually, and provides burn flight teams to ensure safe military aeromedical transfer from the initial hospitalization site to Fort Sam Houston. The staff also conducts clinical burn training programs for physicians, nurses, and allied health professionals and supports clinical trauma research programs. Recently, the Institute's Burn Center and BAMC's Trauma and Critical Care Services was combined to form the Department of Defense's only Trauma Division. This service admits over 1,600 trauma patients yearly, and with this combined effort, a unified approach to trauma and trauma research can now be accomplished.

The Institute's Combat Casualty Care research mission encompasses six basic research areas: Hemostasis, Resuscitation, Bone Tissue Injury, Soft Tissue Injury, Trauma Informatics, and Clinical Trauma. These six areas focus on saving the soldiers' lives, preventing viable tissue loss, and returning the soldiers back to duty as soon as medically possible.

In hemostasis research, the Institute is developing new hemostatic field dressings and tourniquet use for compressible bleeding, intracavitary agents to stop noncompressible bleeding, injectable drugs to enhance or restore hemostatic function, and new devices to stop severe internal bleeding.

In resuscitation research, the Institute is evaluating when,

how, and what kind of resuscitation fluids to use on the battlefield, attempting to standardize treatment by the medic and reduce the amount of fluid the medic has to carry without increasing morbidity or mortality.

In bone tissue injury research, the Institute is studying antimicrobial external fixator pins, antimicrobial bone replacement material, and wound irrigation techniques and devices to reduce morbidity on the battlefield.

In soft tissue injury research, the Institute is investigating antimicrobial polymer bandages, improved field tourniquet, and antibiotics for far-forward use to reduce the impact of tissue injuries and enable soldiers to continue on with their mission.

In trauma informatics research, the Institute is evaluating what to measure and how to develop the medical monitoring devices capable of providing critical real-time information about the severity of wounds and risks of mortality in order to assist the medic in determining the best strategies and priorities for remote trauma triage of injured soldiers on the battlefield.

In clinical trauma research, the Institute is examining a variety of combat casualty care problems in trauma/burn patients including those described in the other five research areas to improve treatment and reduce morbidity and mortality.

The guiding strategy for the Institute's research program is to take the clinical problems identified on the battlefield into our research laboratory for further investigations and solutions, and then validate those solutions in the clinical setting before they are returned to the battlefield as medical doctrine.

With a state-of-the art facility of over 140,000 square feet and a multidisciplinary staff of over 250 personnel, both military and civilian, including surgeons, anesthesiologists, pathologists, nurses, microbiologists, physiologists, biochemists, veterinarians, and technical and administrative support personnel, the USAISR is a world-class organization, well suited to provide combat casualty care solutions for the Department of Defense and the civilians of South Central Texas.

The USAISR has recently supported Operation Iraqi Freedom by deploying surgeons and nurses into theater, training over 1,200 deployed personnel, establishing a semiautomated nationwide daily burn bed availability system, providing logistical support, creating a widely utilized Army Knowledge Online distance learning website, assisted in fielding three new hemorrhage control products on the battlefield, and received every significant burn casualty from the conflict. These efforts highlight the amazing depth and breadth of the military and civilian staff of the USAISR. Our mission is relevant and highly supported by BAMC, MRMC, and our Surgeons General. The personnel assigned feel compelled to accomplish our mission. I am constantly amazed by the quality of the personnel working here and am proud to be a part of the USAISR team.

HISTORY OF THE UNITED STATES ARMY INSTITUTE OF SURGICAL RESEARCH

The Institute of Surgical Research (ISR), originally named the Surgical Research Unit, was established in 1943 to evaluate the role of the newly discovered antibiotics in the treatment of war wounds. The unit was stationed at Halloran General Hospital, Staten Island, NY.

The Institute became a permanent unit and moved to Brooke General Hospital, Brooke Army Medical Center (BAMC), Fort Sam Houston, TX, in 1947, and had 12 personnel assigned. In addition to the study of antibiotics, the unit was also charged with the study of innovative new surgical techniques and developments. In 1949, the unit's mission was expanded to encompass the study of thermal injury due to concern regarding the large number of possible casualties generated by nuclear weapons. The advent of improved grafting procedures and continued use of antibiotics in new applications grew along with this mission.

In May 1953, the unit became a class II activity of the Surgeon General. The unit was assigned to Headquarters, United States Army Medical Research and Development Command in September of 1958. Research flourished, with the Institute evaluating the use of plasma extenders, grafting and preservation of blood vessels, and the use of an "artificial kidney," among other forward thinking medical research initiatives. As the "Army's Burn Unit," this unit has served as a prototype and model for burn units all over the world. During this time, it was also a premier dialysis research center serving South Central Texas and neighboring states.

As part of the Army Medical Department reorganization in March 1994, the Institute became a subordinate command of the Medical Research and Materiel Command, itself a major subordinate command of the newly formed U.S. Army Medical Command and in 1996, the Institute moved to its current location adjacent to the newly constructed BAMC. At this time, the research focus of the mission changed from thermal injury to the full spectrum of combat casualty care. In April 2002, the Institute was placed under operational control of the Commanding General of Brooke Army Medical Center. In April of 2003, the ISR Burn Center and BAMC's Trauma and Critical Care Service were combined to form the DOD's only Trauma Division, under the direction of the Commander, USAISR.

The ISR is a highly decorated and celebrated unit. The Institute has been involved in humanitarian missions overseas to include the USSR in 1989, Guam in 1997, and Honduras from 1999 to present. The unit utilized its expertise by caring for burn casualties from every conflict since WWII to the present Operation Iraqi Freedom, including the 1979 Marine Base fire in Camp Fuji, the 1983 bombing in Beirut, and the Pope AFB paratroopers plane crash in 1994 as well as dozens of other medical emergencies.

The Institute has grown from a 12 person staff to over 250 military and civilian personnel. It continues to serve as the primary Combat Casualty Care research facility for the Army. While continuing its excellence in the field of burn care management, the Institute has expanded and placed equal emphasis in providing medical solutions for the injured soldier on the battlefield.

USAISR's Clinical Burn Center

COL D.J. Barillo, MC, USAR†
COL J.H.Winfree, AN, USA††
E. Greenfield†††

Introduction

In 1949, the Surgical Research Unit (SRU) at Brooke General Hospital was charged with the study of thermal injury and became one of only two burn centers in the U.S. The SRU would be the precursor for today's U.S. Army Institute of Surgical Research (USAISR). Originally focused on antibiotics and adjunctive agents in the treatment of war wounds, the Institute's research focus shifted after WWII to the challenges of patients with multifaceted problems resulting from burn injuries. Since then, the Institute's attention has evolved also to include the management of combat casualty care and traumatic injuries. In April 2002, the Institute was placed under operational control of the Commanding General of Brooke Army Medical Center (BAMC), and in April of 2003 the USAISR Burn Center and BAMC's Trauma and Critical Care Service were combined to form the DOD's only Trauma Division, under the direction of the Commander, USAISR. This collaborative effort will facilitate growth and support intensive research efforts related to battlefield injuries and pre-hospital care, and improve care in the Institute's clinical care activities.

Clinical Activities

The USAISR Burn Center is responsible for the day-to-day care of severely injured burn patients. Care at the USAISR includes the initial evaluation and clinical management of these patients, the surgical care of the patients, and completion of medical records. The Burn Center is a state of the art facility consistings of two 8-bed intensive care units (ICUs), a 24 bed step-down unit, an operating room suite, a fluoroscopic suite, a metabolic kitchen, a physical (PT) and occupational therapy (OT) gym, and office space for the burn team. The two ICUs are designed as mirror images. For normal Burn Center operation only one ICU is required. The second ICU serves as overflow capacity for use in mass-casualty incidents, or for infection control purposes. Because each room in the 24-bed step down unit is configured with oxygen, suction, and cardiac monitoring, a total of 40 ICU patients can be treated if necessary. A multidisciplinary outpatient ambulatory burn care provides consultative and follow-up care to eligible outpatients. In addition, it participates in institutional research and review of treatments and technology. The Center provides telephonic and limited on-site consultative services to all military hospitals in the U.S. and abroad, and civilian hospitals in the greater San Antonio area. Also provided are PT, OT, psychiatric, and referral to social services to each patient as needed. In 2002, the overall clinical workload of the Burn Center included 234 admissions, 569 operative and endoscopic procedures, and 786 patient visits to the burn clinic.

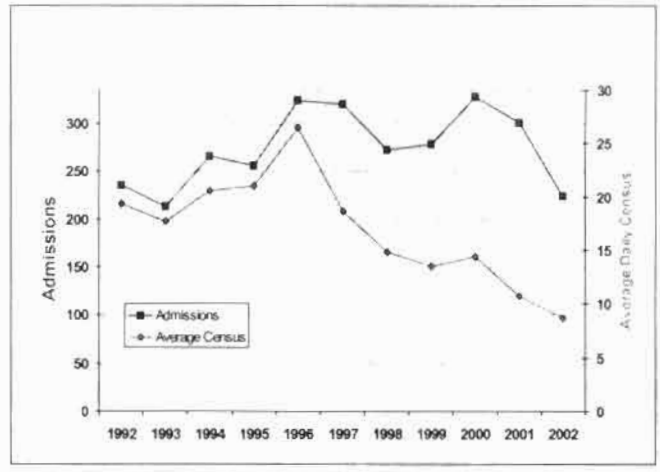


Fig 1. Yearly admissions and average daily census.

The Burn Center also instituted its own Burn Flight Team to transport patients to the Center both nationally and internationally. The teams provided professional medical augmentation (technical advice and support) to local medical authorities in triage, treatment, stabilization, care, and evacuation of burn patients associated with disaster/mass-casualty incidents. Since its inception in 1953, the Burn Flight Team has transported over 2,500 burn patients in the conduct of its worldwide mission. Some of the more notable missions include the aeromedical transport of 40 Marines injured in a barracks fire in Camp Zama, Japan, in 1979 and the massive natural gas explosion in Ufa, Russia, in June 1989, when as directed by the President, George Bush, the Institute provided a 21-member burn team to assist in the care of over 700 injured. During Operation Desert Shield/Desert Storm, the USAISR established three burn teams in Saudi Arabia and a burn holding unit at the U.S. Army Regional Medical Center in Landstuhl, Germany. More recently, in March 1994, USAISR received 43 of the more than 100 active duty soldiers who were injured or killed during the Fort Bragg and Pope Force Base midair collision disaster. Four flight teams were deployed to Fort Bragg to transport these patients to the USAISR and one team flew to

Chapel Hill to transfer three additional soldiers who had been sent there during initial triage. Other major aeromedical transfer missions include responding to disasters in Korea, at Ramstein Air Base, and onboard the USS Midway.

Fig 2. Burn flight teams.

Today, these aeromedical teams have evolved into the Burn Special Medical Augmentation Response Team (B-SMART). The smart mission is domestic and international disaster response, especially in cases of terrorism and weapons of mass destruction. They provide world-class medical augmentation in burn and trauma triage, resuscitation, treatment, and evacuation. They are prepared to deploy within 12 hours of notification. During the 60 days surrounding the 2002 Winter Olympics in Salt Lake City, UT, the B-SMART teams remained on-call.

Research Activities

A number of landmark studies and innovations in the care of burn and trauma patients have been developed at the Institute of Surgical Research (ISR). Early research at the Institute pioneered the development of formulae for burn resuscitation, effective topical antimicrobial therapy, prophylaxis against stress ulceration of the GI mucosa, and nutritional repletion of the burn patient. The success of both the clinical operation and the research effort derive from a team approach to burn care that is truly multidisciplinary. Recent innovations have also been made in the areas of respiratory therapy and OT/PT.

The Respiratory Therapy Section has pioneered the use of high frequency percussive ventilation for the treatment of patients with smoke inhalation injury.[1,2] Studies of this modality at USAISR demonstrated a significant decrease in the incidence of pneumonia along with a significant increase in patient survival when compared to historical controls.[2] Because the Respiratory Therapy section also provides personnel to the Burn Flight Team, significant effort has been undertaken to find the optimal method of providing in-flight ventilation for burn patients. A follow-up study utilizing a different portable ventilator designed to provide both pressure control and high frequency ventilation is in progress with promising preliminary results.[3]

A number of studies have been carried out on the prevention and treatment of contractures following burn injury. The ISR was among the first to describe the utility of computer-assisted evaluation of hand and upper extremity function.[4] A series of standard multidisciplinary treatment pathways for patients with burned hands was developed and the innovative use of unusual flap procedures for the coverage of difficult hand burns has been described.[5,6]

The ISR Burn Rehab OT/PT section is also improving burn rehabilitation with state of the art technology. Along with the University of Texas Health Science Center's Physical Medicine Department at San Antonio, they have developed an integrated system of computer-aided design and computer-aided manufacturing to fabricate transparent facemasks for the treatment of facial scarring. Compared with conventional methods of mask fabrication, this system is faster, more accurate, and less stressful for the patient, and allows for greater control of the finished product.

A number of prospective studies have recently been developed into several planned clinical trials. At this time, studies are in advanced planning to study the efficacy of using a nasal spray of ketamine for the relief of pain and using liquid dressings as a protective cover of skin graft donor sites. Both of these studies have a direct application to trauma medicine on the battlefield. Other clinical studies under consideration are developing algorithms for remote triage, deciding which is the best fluid for resuscitation on the battlefield, and evaluating oxygen carriers for infusion.

Educational Activities

The interdisciplinary staff at the ISR provides a biannual 3-day burn care seminar followed by a 2-day skills fair. This seminar initially served to orient newly assigned nursing personnel. Presently, the seminar is available to the general public offering 26 hours of continuing medical education and continuing education units. Advanced Burn Life Support (ABLS) is also presented twice a year and is attended by hospital staff and the community. The USAISR created and maintains a presence on Army Knowledge Online (AKO). References available include: SMART Burn Team Training, lectures on Combat Casualty Care, Combat Burn Life Support

(CBLS) Training as developed by the USAISR, Civilian ABLS supplied by the American Burn Association (ABA), Combat Trauma Registry supplied by the AMEDD Center and School, and the Emergency Warfare Surgery Handbook disks (CD) including ABLS, the draft versions of the Emergency Warfare Surgery Handbook. The USAISR site has become 32d in the top 100 Army Communities with files for download (both private and public) and 86th in the top 100 Army Communities by downloads as of 21 April 2003. (**https://www.us.army.mil/portal/portal_home.jhtml** [After login, navigate sequentially through the options to: (1) Collaborate, (2) Army Communities, (3) MEDCOM, (4) MRMC, and (5) USAISR]).

Supporting Operation Iraqi Freedom (OIF) and the Global War on Terrorism

In anticipation of armed conflict in the Middle East, the Army assembled a planning group in the Fall of 2002. It was recognized that a center for vesicant casualties needed to be established. Fortunately, the expertise to manage this injury already existed within two sister units within the Medical Research and Materiel Command: the USAISR and the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD). Collaboration between these units produced a rational plan for the management of chemical (vesicant) casualties at the USAISR. A course covering the burn center management of vesicant injury was developed jointly by the USAISR and the USAMRICD to augment the excellent courses already available through USAMRICD on the battlefield management of such injuries.

As part of this exercise, need for the training of in-theatre medical personnel in the basics of burn care also was identified. A standard burn care course, ABLS, developed by the ABA has been in use by the U.S. military since the 1980s. This course is intended primarily for a civilian audience, but also addresses the unique needs of a deployed military force. However, for this reason, several additional teaching modules were required. Thus, CBLS was developed by USAISR personnel to address the unique needs of the military to include information on initial management of burns, vesicants, and triage/aeromedical evacuation. In support of OIF, the Institute developed and implemented a plan for worldwide training of deployed and deploying personnel. This training was offered to the entire BAMC staff and other community medical treatment facilities in anticipation of receiving war casualties with burn and vesicant injuries. From January – April 2003 the USAISR staff trained approximately 1,200 individuals on ABLS and CBLS.

Just before the start of OIF, USAISR burn surgeons were deployed to both Southwest Asia and Europe. These personnel provided consultation and evacuation triage capability to the European theater of operations and facilitated evacuation and routing of burn patients. The Burn Surgeon Liaison Officer's, as part of the B-SMART teams, also provided essential burn/vesicant care training for deploying/deployed units and medical personnel in the European Theater. At home, the USAISR expanded its clinical facility from 20 to 40 burn beds and created a semi-automated nationwide daily burn bed availability system in coordination with the ABA. This system provided current burn bed availability information to the liaison officer burn surgeons who then provided recommendations to medical evacuation regulators and TRANSCOM as to which hospital the casualty should be sent. As previously mentioned, the USAISR also created and maintained the knowledge centers within AKO for worldwide reference availability.

Conclusion

Building on the accomplishments of the past but always looking toward the future, the USAISR benefits not only the burn patient, but all injured warfighters on the modern battlefield. The integration of clinical care and laboratory research at the Institute has been extremely fruitful in this endeavor, and researchers at USAISR have produced over 900 publications from all disciplines in peer-reviewed journals as well as multiple chapters in surgical and burn care textbooks. The alumni of the institute include over 86 full professors of academic departments, 26 chairs of academic departments, and at least 20 directors of other burn centers. The improvements in care and achievements in research have firmly established the international stature of the Institute. The daily operations of the Burn Center have provided it with the experience, expertise, and capability to meet the demands of any mass casualty situation on literally a moment's notice, whether it is a civilian disaster or a military operation in the global war on terrorism, such as OIF. The USAISR will continue to sharpen its leading edge in clinical care, training, and research activities, and in doing so, further improve the outcomes of patients with thermal and traumatic injuries.

References

1. Cioffi WG, Graves TA, McManus WF, Pruitt BA. High frequency percussive ventilation in patients with inhalation injury. *J Trauma*. 1989;29:350-354.

2. Cioffi WG, Rue LW, Graves TA, McManus WF, Mason AD, Pruitt BA. Prophylactic use of high frequency percussive eventuation in patients with inhalation injury. *Ann Surg*. 1991;213:575-582.

3. Galvez E, Park MS, Harshbarger TL, Jordan BS, et al. Evaluation of the Duotron, a high frequency pressure cycled transport ventilator. *J Burn Care and Rehabilitation*. 1999; 20:1, part 2, p S201.

4. Barillo DJ, Craigie JE. Burn injury. In: Hurd WW, Jernigan JG. eds: *Aeromedical Evacuation: Management of the Acute and Stabilized Patient.* New York: Springer-Verlag; 2002.

5. Harvey KD, Barillo DJ, Hobbs CL, Mozingo DW, et al. Computer-assisted evaluation of hand and arm function after thermal injury. *J Burn Care and Rehabilitation.* 1996;17:176-80.

6. Barillo DJ, Arabitg R, Cancio LC, Goodwin CW. Distant pedicle flaps for soft tissue coverage of severely burned hands: an old idea revisited. *Burns.* 2001.

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Development of the SMEED™ Platform

LTC Leopoldo C. Cancio, MC, USA†
SGT William VanPutte††

Introduction

The U.S. Army Institute of Surgical Research (USAISR) has a long history of innovation in the field of aeromedical evacuation technology and doctrine. The Institute pioneered the long-distance aeromedical evacuation of burn casualties during the early 1950s. More recently, the Institute has served as a test-bed for transport mechanical ventilators and other critical care technology.[1] The Special Medical Emergency Evacuation Device (SMEED™) platform is another example of a technology which developed because of synergy between a real-world combat casualty care mission, and a product-oriented research team.

In Mar 00, the Institute's Special Medical Augmentation Response Team for Burns (SMART Team) conducted a training exercise aboard a C17 aircraft at Kelly AFB, TX. The exercise involved the simulated aeromedical transportation of 10 critically ill, mechanically ventilated casualties. During that exercise, it was evident that the lack of a suitable method (other than multiple litter straps) of securing monitors, ventilators, suction, and similar equipment to patient litters resulted in significant confusion, loss of time, and potential for damage to equipment and/or injury to patients. An Institute Noncommissioned Officer, SFC Eric Smeed, volunteered to develop such a technology. The requirements established were:

- Rugged
- Lightweight
- Inexpensive
- Compatible with existing and future medical technology
- Customizable to meet individual needs and equipment portfolios
- Approved for use aboard U.S. Air Force (USAF) and U.S. Army aircraft

Design Process

Sergeant First Class Smeed designed a succession of four prototype platforms to meet these needs, directing their fabrication at the Air Force Research Laboratory, Brooks AFB, TX and, later, at a local machine shop in San Antonio, TX. The basic design concept (figure) features an aluminum and steel platform which clamps onto the side poles of the NATO litter. Individual equipment items are then attached to the platform by means of additional mounting brackets.

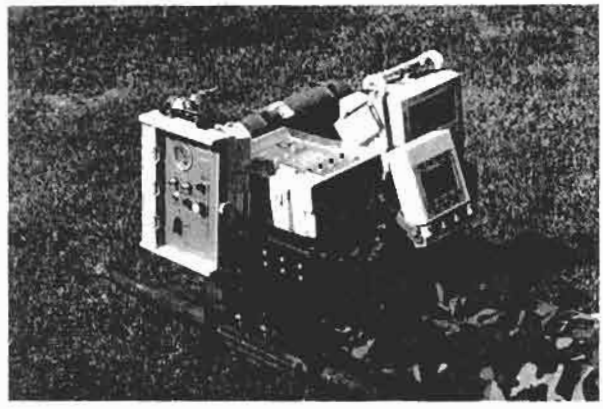

Fig. SMEDD™ platform.

Essential to the design process was close collaboration between SFC Smeed, the machinists, and rigorous field testing by the SMART Team. This included exercises at the Soldier-Medic Training Site, Camp Bullis, TX, and at the Florida Ranger Camp, Eglin AFB, FL. The platform was then submitted to the Air Force Medical Equipment Development (AFMED) laboratory at Brooks AFB, TX, for flight-testing. The total time between identification of the problem in Mar 00 and USAF flight approval of the first prototype was 9 months. The total cost for construction of this prototype was $1900, and the cost for its flight-testing was $2346.

The platform then underwent further revision at the USAISR. Whereas the initial prototype was designed for USAISR SMART Team use, we now sought to develop a platform that would accommodate all of the standard equipment items in use across the military. The final prototype has the following specifications:

- Composition: Aluminum sheet and stainless steel parts
- Color: Anodized flat black
- Weight (empty): 20 lbs
- Dimensions:
  - Length: 14"
  - Width: 22"
  - Height: adjustable to three different levels above

the patient, with height measured at the top of the litter pole to the inside of the top plate
Low: 5 1/16"
Middle: 7 9/16"
High: 10 1/16"

- Mounts on the NATO litter at any location, head-to-toe (excluding handles)
- Accommodates any reasonable medical equipment load
- Compatible with all USAF and U.S. Army airframes, to include the UH60
- Folds to a briefcase-like package

The following equipment has been successfully mounted onto the platform:

- Uni-Vent® Eagle™ Model 754 Portable Ventilator (Impact Instrumentation, Inc)
- Ultra-lite® Model 326 Portable Aspirator (Impact Instrumentation, Inc)
- Military Transporter ventilator (Percussionaire, Inc, Sandpoint, ID)
- Propaq Encore™ Monitor (Welch Allyn Protocol, Inc, Beaverton, OR)
- MedSystem III® Infusion Pump (Alaris Medical Systems, Inc, San Diego, CA)
- Steel oxygen cylinders (size "D")
- Carbon-fiber oxygen cylinders (size "EE")
- Lifepak 10 debrillator (PhysioControl, Inc, Redmond, WA)

This new version successfully underwent AFMED testing as well, to include vibration tests to jet, turbo-prop, and helicopter characteristics. The SMEED™ passed all tests successfully and was approved for use during all phases of flight on all USAF aircraft (including fixed and rotary wing). Additional comments from the AFMED were: "The SMEED design offers maximum flexibility in securing medical equipment devices needed for patient care directly on the patient's litter. This improvement allows continuous monitoring, patient care and comfort, and may reduce the need for an equipment litter. The SMEED is an important advancement in aeromedical equipment securing technology by accommodating a variety of Patient Movement Items common to all military services. Testing by the U.S. Army Aeromedical Research Laboratory, Fort Rucker, AL, is scheduled for this fiscal year.

Presentations

Communication with potential users has been an essential component of the successful development process for the SMEED™. In addition to the Institute's own SMART Team, the device has been briefed to customers in the Air Force, Army, Navy, Coast Guard, Marine Corps, Special Operations, and civilian communities. This educational effort has included presentations at the following conferences:

- Advanced Technology Applications for Combat Casualty Care conference, Fort Walton Beach, FL, Sep 00 and Sep 01; St Petersburg Beach, FL, Sep 02.
- Special Operations Medical Association conference, Tampa, FL, Dec 00-01.
- U.S. Army Medical Research and Materiel Command 2001 Acquisition, Logistics, and Technology conference, May 01.
- Sea-Air-Space Conference, Washington, DC, Mar 02.
- Critical Care Transport Medical Conference, Las Vegas, NV, Apr 02.
- Association of the United States Army Medical Symposium and Exhibition, San Antonio, TX, May 02.
- Army Aviation Association of American Annual Convention, Nashville, TN.
- National Guard Association of the United States Conference, Long Beach, CA.

Further Evaluation and Manufacturing

Ongoing evaluation of the system has included inclusion in several major exercises. In Jan 02, the Air Force Medical Equipment Support Activity, Fort Detrick, MD, conducted Military Utility Assessment of the SMEED™ during the Civil Reserve Air Fleet (CRAF) exercise. The CRAF is a mechanism that enables the USAF to use civilian passenger aircraft as aeromedical evacuation platforms during a national emergency. Then, in Jul 02, the platform was evaluated at the Joint Service Seahawk exercise, during which it was used on the C130, C9, and C17 aircraft, as well as on a variety of ground vehicles.

The final ("version 4") prototype has been patented (Ref U.S. Patient No. 6493890) and has been transitioned to advanced product development by the U.S. Army Medical Materiel Development Agency (USAMMDA), Fort Detrick, MD. Close collaboration with USAMMDA was critical to the success of this phase of product development, and led to licensing by Impact Instrumentation, Inc (West Caldwell, NJ) to manufacture the SMEED™.

Conclusion

In part, the success of the SMEED™ can be judged by SFC Smeed winning the Fort Sam Houston Suggestor of the Year award for 2002 on 25 Apr 02 and the U.S. Army Suggestor of the Year award for 2002 on 14 Mar 03. An even more satisfying accomplishment for the product team has been the knowledge that it was deployed during Operation Enduring Freedom in support of U.S. Army Rangers, and is currently deployed in Operation Iraqi Freedom as a component of the U.S. Marine Corps Forward Resuscitative Surgical System.

In reflecting on the reasons for the rapid success of this project, several conclusions come to mind. First, the SMEED™ platform grew out of a real-world need, expressed and explained by end-users. These end-users were intimately involved in the design process. The design team was compact in size. Unnecessary administrative intervention was minimized. The team was backed up by expertise at the Institute and at the U.S. Medical Research and Materiel Command in intellectual property and advanced product development. Opportunities for making the product relevant to all the branches of the military were aggressively pursued. Finally, the inventive genius of an Army noncommissioned officer was actively encouraged.

Detailed information on the platform is available in a technical report.[2]

References

1. Barillo DJ, Dickerson EE, Cioffi WG, Mozingo DW, Pruitt BA, Jr. Pressure-controlled ventilation for the long-range aeromedical transport of patients with burns. J Burn Care Rehab. 1997; 18:200-5.

2. Smeed EM, VanPutte WD, Drew G, Cancio LC. Special medical emergency evacuation device platform: life support for the objective force. Fort Sam Houston, TX: U.S. Army Institute of Surgical Research; 2002.

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Overview of the Hemostasis Research Program: Advances and Future Directions

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Introduction

The mission of the Combat Casualty Care Research Program of the U.S. Army Medical Research and Materiel Command is to reduce the morbidity and mortality resulting from injuries on the battlefield through the development of new lifesaving strategies, surgical techniques, biological and mechanical products, and the timely use of telemedicine technologies. One of the major areas of focus of the Combat Casualty Care Program is hemorrhage control. This article provides an overview of the Hemostasis Research Program (HRP) and its accomplishments to date, and makes suggestions for areas of basic research in the future. The HRP is based at the U.S. Army Institute of Surgical Research (ISR) and includes collaborations with extramural research laboratories.

The Need: Decreased Combat Mortality

Due to improvements in survival after evacuation from the battlefield, overall mortality from combat wounds has decreased during the past hundred years. Although mortality has decreased for those dying of wounds after evacuation, there has been little improvement in the number of those killed in action (KIA) (Figure 1). Furthermore, the percentage of the wounded that die on the battlefield increases with prolonged evacuation, an increasingly likely scenario under the Objective Force as troops are more dispersed throughout the combat arena.[1]

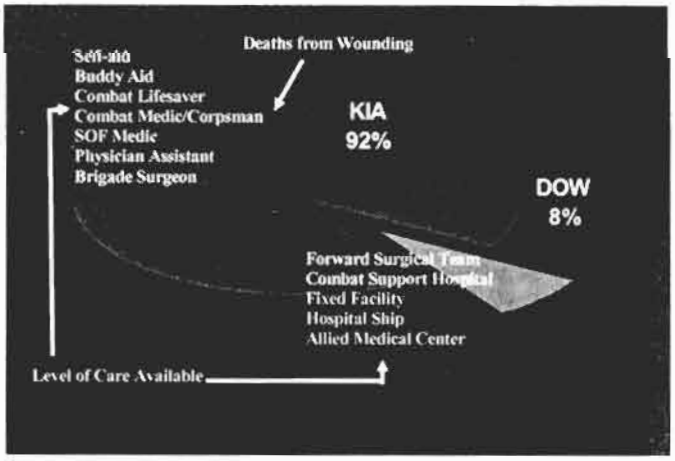

Fig. 1 Level of care available pre- and post-evacuation.

Hemorrhage is the leading cause of death from wounds on the battlefield (accounting for over 50% of deaths) and the second leading cause of death in civilian trauma.[1,2] Although some soldiers killed on the battlefield are clearly unsalvageable and become KIA within minutes of impact and, it appears that approximately one-third of KIA would be salvageable (Figure 2) with the development and fielding of new methods for early intervention.[3] Data supporting the position that a salvageable population of KIA exists were obtained in Oman in 1973 and Panama in 1989.[4,5] In both instances, the stationing of emergency medicine physicians at casualty collection points provided advanced medical care closer to the point of wounding and resulted in lower KIA rates than in previous conflicts. Given the possibility of a significant population of salvageable KIA, the hemorrhage control program has focused primarily on providing new methods, drugs, or devices to those present or near the point of wounding, such as the wounded soldier himself, his buddy, the combat lifesaver, or the combat medic.

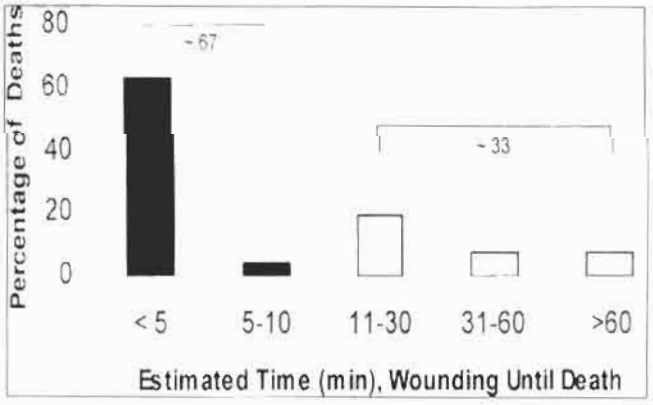

Fig. 2. Percentage of deaths on the battlefield occurring at different times from wounding in Vietnam. Making the assumption that deaths occurring within the first 15 minutes are not salvageable, approximately 33% of KIA deaths are potentially salvageable. Adapted from (see Reference No. 3)

Hemostasis Research Program Objectives

Figure 3 depicts the current state of field hemorrhage control and the objectives we strive to meet by 2010. Approximately 20% of hemorrhagic deaths are due to compressible wounds (those that are accessible to direct pressure), treatable with pressure dressings, tourniquets, and mechanical surgical methods. However, the vast majority (approximately 80%) of hemorrhagic deaths on the battlefield are due to intracavitary hemorrhage, which is not accessible for direct compression (for example, within the pelvic, abdominal, or thoracic cavities). Currently, no method other than surgical intervention can treat intracavitary hemorrhage. The mission of the HRP is to develop procedures, devices, or agents that may be used by the soldier himself, a buddy, a combat medic or higher echelon medical personnel to control compressible and noncompressible hemorrhage under far-forward situations. We will therefore discuss in subsequent sections the devices, drugs, and methods that are currently being developed or evaluated to accomplish this objective.

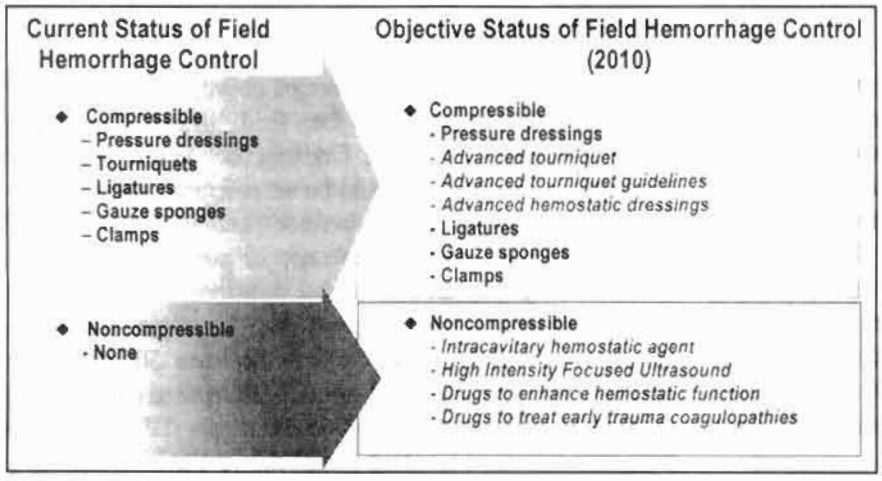

Fig 3. Current (2003) and future (2010) field hemorrhage control.

One-Handed Tourniquet. One need identified by the soldier in the field has been a tourniquet that can be self-applied by a wounded soldier with one hand. Project development was originally funded by the U.S. Special Operations Command (USSOCOM), but was subsequently "handed off" to the HRP for further development. To this end, Calkins et al developed a needs survey that asked Special Operations corpsmen to rank desired characteristics in a tourniquet.[6] A comprehensive search of commercially available tourniquets or patented tourniquet designs was subsequently performed and several novel tourniquet designs were also developed. These tourniquet designs were then evaluated as to how well they met the desires of the user community as expressed in the survey, as well as how well they performed under austere far-forward conditions. Special Operations medics then tested seven tourniquet designs for successful tourniquet placement and time required for placement. Preliminary field-testing results suggested a ratchet design as the best tourniquet available for field use. It was later recognized that this was not the optimal design.[6]

Subsequently, two new prototype designs (cinch and wrap) were developed and modifications were made to the ratchet design by investigators at the Walter Reed Army Institute of Research. These three designs were then evaluated based on their effectiveness, application time, size, and weight. Furthermore, laboratory testing on artificial limbs assessed the pressure distribution resulting from the tourniquet application. An ad hoc panel containing both scientists and combat medics then convened at the ISR, and this panel concluded that the cinch design best met the requirements of the user community. With slight modifications, this tourniquet was produced and sent to field users (USSOCOM, AMEDD Center and School, U.S. Navy, and Marine Corps) for ongoing evaluation. Subsequently, a manufacturer was found and large-scale manufacturing was begun.

Special Forces medics in Afghanistan and Iraq are currently using the one-handed tourniquet. Additionally, an expert panel is being convened to promulgate guidelines for field use of the one-handed tourniquet as well as to address the potential for field removal of the tourniquet if used in combination with advanced hemostatic dressings. Finally, ongoing testing of the one-handed tourniquet in the uninjured limbs of healthy volunteers will quantitatively determine the actual blood flow reduction produced by its use.

Advanced Topical Hemostatic Dressings. Under the auspices of the HRP, a variety of topical hemostatic dressings have been tested for their potential applications to trauma using both severe liver injury and arterial injury models in swine.[7-9] As a result of these evaluations, two dressings are currently approved for external use in the field, the American Red Cross Dry Fibrin Sealant Hemostatic Dressing (under an IND protocol) and the Food and Drug Administration (FDA)-approved HemCon Chitosan bandage. The ability of these bandages to act as the primary hemostatic mechanism for internal use (in lieu of surgical intervention) over prolonged periods of time (for example, in the event of extended evacuation) is currently being tested at the ISR. For a detailed description of the development and evaluation of these dressings, please see the article by Kheirabadi et al in this issue.

Intracavitary Hemostatic Agent. A major issue to be addressed by the HRP is a method to control bleeding from

noncompressible truncal wounds, for which surgical intervention is currently the only effective treatment. The concept of an intracavitary hemostatic agent was advanced to treat such an injury in the field. The hypothesis was that a hemostatic material could potentially be infused into a closed body cavity by a trocar, spread throughout the closed cavity, and interact with the bleeding sites to stop hemorrhage. In the gunshot wound, for example, foam could be delivered through the hole left by the penetrating object and administered close to the injury site to reduce internal bleeding. In order for this to be a viable option, however, a putative intracavitary hemostatic agent must: (1) produce hemostasis without applied pressure when administered directly to an actively bleeding site; (2) distribute uniformly throughout the body cavity when it is introduced into a closed cavity and stop hemorrhage upon contact with actively bleeding tissues. In addition to providing hemostatic efficacy, it has been proposed that an intracavitary hemostatic agent might also provide enough intracavitary pressure to provide some level of tamponade, thereby aiding in hemorrhage control. It is important to emphasize, however, that intracavitary pressure cannot be increased to the point at which function of vital organs is compromised.

Initially, several liquid hemostatic agents were tested in internal hemorrhage models in small animals to evaluate their ability to provide hemostasis without applied pressure. Surgical liquid FS (for example, was transformed into a foam and sprayed directly onto the bleeding surface of a lacerated liver in rodents). Although an encouraging decrease in blood loss was observed, application of the FS foam did not increase survival rate 1 hour post-application.[10] Furthermore, experimentation with the same formulation of FS foam in a rabbit model of partial liver resection could not replicate the reduction in blood loss seen in the rat liver injury model (Kheirabadi, unpublished data).

In subsequent work, the formulation of the active components of FS was substantially modified to provide a highly adhesive fibrin foam that would attach firmly to rabbit liver slices even if the tissues were covered with fresh blood. In rabbits, direct application of this modified FS foam on the bleeding surface of the lacerated liver was partially effective in reducing the hemorrhage and improving survival. Infusion of the FS foam into a rabbit model of closed-abdomen bleeding, however, had only a marginal effect in reducing hemorrhage or improving survival rate. In other studies using a variety of means of making the foam (for example, chemical, propellant gas), FS foam did not prove to be effective in reducing bleeding under practical application circumstances (without producing large increases in intracavitary pressure that disrupted vital organ function).

Currently, we are in the process of testing other potential hemostatic agents that might be used as intracavitary agents. Although not a foam, FloSeal (Baxter Biomedical) consists primarily of collagen and thrombin. When applied directly to the bleeding liver injury in the open abdomen rat model, FloSeal was able to reduce blood loss by almost 25%, but did not significantly increase survival time. In a closed-abdomen model of internal hemorrhage, however, FloSeal neither reduced blood loss nor improved survival time in rats.

There are two major obstacles to the effectiveness of any such agent when infused into a closed body cavity. The first is the inability of these agents to distribute thoroughly in the cavity and reach the entire injured surfaces before they become activated. The second major obstacle is the inability of these agents to penetrate through the pooled blood around the organs and move against free-flowing blood to interact with the injured tissues themselves. Hence, although intracavitary foams/liquid agents offer an alluring solution to hemorrhage from noncompressible wounds, there are as yet many obstacles to overcome and it is unclear at this point whether this potential solution to noncompressible hemorrhage is possible using current technology.

Hemostatic Drugs. As indicated above, there is currently no treatment available to the field medic for severe noncompressible hemorrhage. One approach to this problem is to identify a drug(s) that could be administered intravenously that might act systemically to decrease bleeding. The concept of using intravenous drugs to enhance or augment the body's innate clotting mechanisms during situations in which blood loss is expected is not new. Indeed, drugs have been used in the treatment of bleeding complications for over 30 years. For example, the FDA-approved drugs epsilon-amino caproic acid, tranexamic acid, aprotinin, and desmopressin (DDAVP) have been used to reduce bleeding complications and blood loss in a variety of clinical situations including cardiac surgery, hepatic surgery, oral surgery, knee and hip arthroplasty, and in patients with bleeding disorders.[11] More recently, recombinant factor VIIa (rFVIIa) has been used very effectively in hemophiliac patients for controlling acute bleeding episodes and during surgical procedures.[12]

Another objective of the HRP is to screen these FDA-approved drugs for their potential use in noncompressible traumatic hemorrhage. Preliminary results suggest that epsilon-amino caproic acid, tranexamic acid, aprotinin, or DDAVP, when used alone, neither decreases bleeding time nor increases survival time following severe liver injury in rodents (Ryan et al in preparation). Further studies are ongoing to determine whether combinations of such drugs might be effective in this severe liver injury model.

A more promising candidate to provide hemostasis

following trauma is rFVIIa. A growing number of case reports document the successful use of rFVIIa to decrease blood loss in trauma patients.[13] In laboratory models using pigs with normal coagulation function, however, rFVIIa does not appear to decrease blood loss following severe liver injury, aortic injury, or liver avulsion.[14-17] Administration of rFVIIa in these pigs does, however, activate clotting mechanisms and increases the pressure at which rebleeding occurs during resuscitation, suggesting that rFVIIa may strengthen the nascent clot.[15,16] In pigs with abnormal coagulation function induced by reduction of body temperature (hypothermia) and dilution of blood (hemodilution), rFVIIa decreased the severe blood loss following severe liver injury in two studies.[18,19] It therefore appears that rFVIIa use may be beneficial in the coagulopathic soldier in whom bleeding cannot be stopped by other means. Ongoing laboratory investigations will further delineate the conditions under which this drug might be useful.

In addition to rFVIIa, we are studying the ability of a new drug, factor Xa-PCPS (phosphatidyl choline-phosphatidyl serine vesicles), to reduce bleeding in swine models of trauma. We have established collaborative agreements with investigators at Haematologic Technologies, Inc and the University of Vermont, who developed the drug. In preliminary laboratory tests, this drug stopped cuticular bleeding in both normal and hemophiliac dogs.[20] As other new hemostatic agents are developed, they will likewise be tested in trauma-relevant animal models for their potential to decrease bleeding and to save the lives of wounded soldiers on the battlefield.

Mechanisms of Early Trauma-Related Coagulopathy. As alluded to above, early responses (within 24 hours) to trauma and subsequent resuscitation, especially under battlefield conditions, may include hypothermia, hemodilution, and acidosis. Such conditions induce coagulopathies in which normal coagulation function is altered and disrupted. As part of the HRP, we are investigating trauma-related coagulopathy in a complex setting designed to more closely model combat injuries sustained on battlefields. Our goal is to identify the changes of coagulation activity, platelet function, and fibrinolysis using our existing animal model and techniques, as well as to develop new techniques and understandings of the basic physiological mechanisms underlying the development of coagulopathy.

We are currently studying physiological mechanisms underlying the development of such coagulopathies. In our initial project, we have developed an in vivo swine model to study coagulopathy with hypothermia and acidosis, as well as other in vitro methods using physiologically relevant agonists. One of these in vitro methods is a measurement in minimally altered pig blood that illustrates the clot process over time. We have found that acidosis and hypothermia (alone and in combination) cause significant increases in bleeding time and decreases in fibrinogen concentration and platelet level. These were associated with significant increases in prothrombin time and activated partial thromboplastin time which are measures of clotting via the extrinsic and intrinsic coagulation pathways, respectively. However, platelet function was unchanged when temperature was decreased from 39°C to 32°C. Development of this in vivo animal model and new in vitro methods has expanded our ability to characterize the coagulopathic state in greater detail than that provided by previous investigations. These initial results have provided valuable information about the mechanisms underlying coagulopathy induced by hypothermia and acidosis, and will be expanded to include investigations into coagulopathies associated with hemodilution or combinations of this trauma-induced triad (hypothermia, acidosis, and hemodilution). Our comprehensive approach is aimed towards gaining insightful information and directions for pharmaceutical intervention to correct trauma-related coagulopathies and thereby save lives of wounded soldiers.

High Intensity Focused Ultrasound (HIFU) Device. In addition to screening potential hemostatic agents, the ISR is currently evaluating other means to reduce noncompressible hemorrhage. Ideally, a combat medic or other care provider would have a device to noninvasively visualize a source of internal bleeding and to safely cauterize it. Although this sounds like science fiction, investigators at the Applied Physics Laboratory at the University of Washington are working toward such a device. Led by Dr Larry Crum, these investigators are developing a hand-held device that will (1) incorporate a computerized Doppler system to locate bleeding structures and (2) focus sound waves to cauterize the bleeders without damaging the overlying tissues.[21] So far, acoustic hemostasis has been shown to be effective in sustaining hemostasis (up to 60 days) in exposed splenic lacerations.[22] Currently, these investigators have developed animal models in which blood vessel and liver injuries are made noninvasively. These injuries are subsequently visualized and cauterized using noninvasive methods. The HRP has a collaborative agreement with the Applied Physics Laboratory, thereby leveraging industry and academic investments to meet Army needs. Investigators from the two programs interact on a regular basis and HIFU will eventually be tested in animal models of trauma at the ISR.

Integrated Approaches. It should be emphasized that the hemostatic tools discussed above are not conceived as disparate solutions to the problem of bleeding; rather, the concept is that these tools can be used to complement each other in the field. Figure 4 depicts two scenarios in which the use of hemostatic tools could be integrated. In the first, a soldier with a bullet

wound to the thigh that includes femoral artery injury could be treated at the level of self/buddy aid by applying a tourniquet, an advanced hemostatic dressing, or both. If the tourniquet accomplishes vascular control, an advanced hemostatic dressing might then be applied and the tourniquet released, thereby improving the possibility of limb salvage. The battalion surgeon might then use HIFU to cauterize the arterial injury, if necessary. In the second scenario, a soldier with a shrapnel wound that produces severe liver laceration could be treated by a combat medic using an intracavitary hemostatic agent and/or an intravenously administered hemostatic drug. The battalion surgeon might then opt to use HIFU to cauterize the wound and, if the soldier had developed coagulopathy, additional intravenous agents might be administered to reverse the coagulopathy. The goal of the HRP is to provide an array of advanced hemostatic tools to care providers, thereby allowing greater flexibility in patient treatment based on immediate clinical/combat demands.

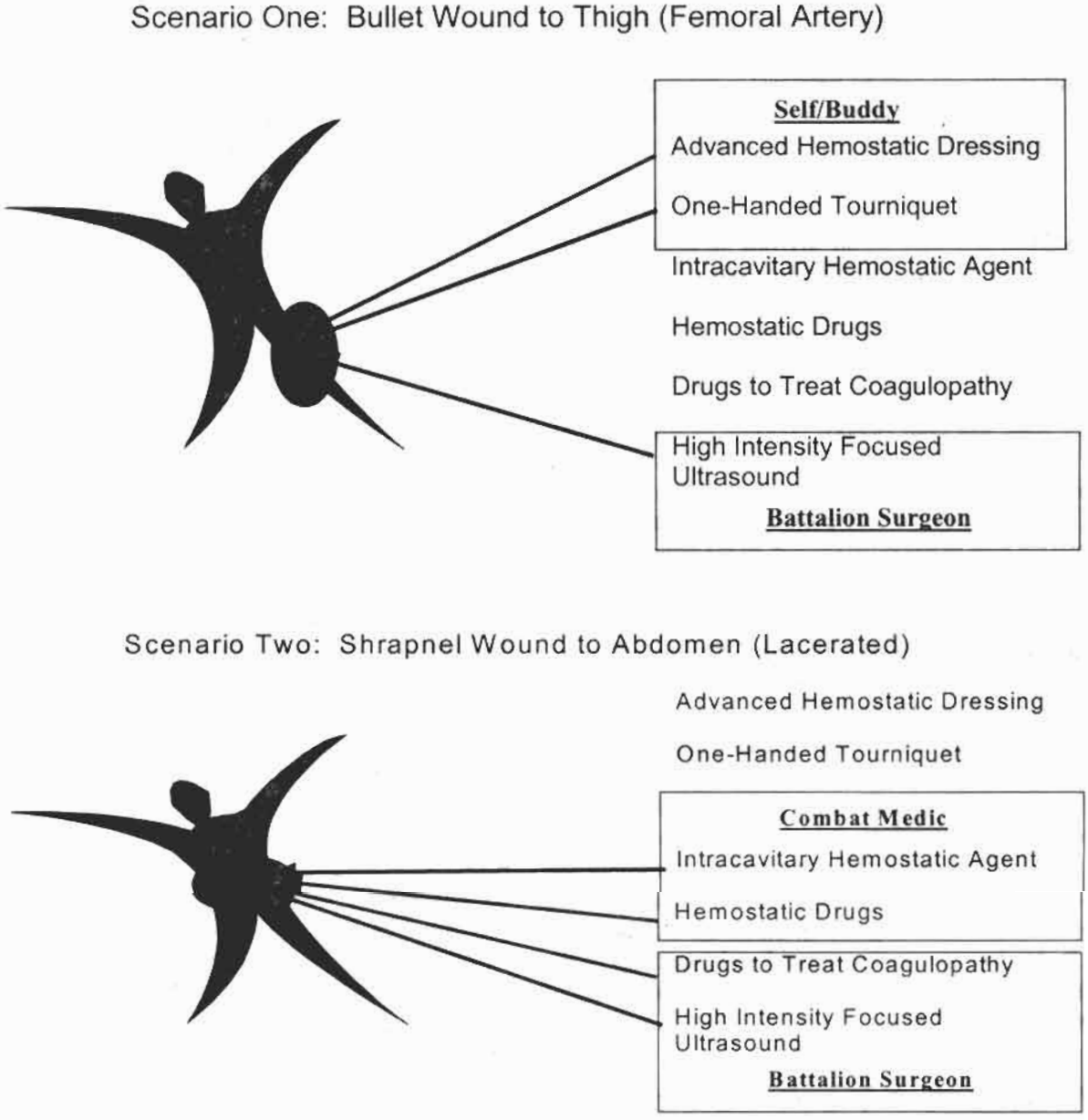

Fig 4. Possible scenarios for integrated use of hemostatic tools. Current pre-evacuation treatment for scenario one is gauze dressing and use of field expedient tourniquet or belt. For scenario two, there is currently no efficacious pre-evacuation treatment available.

Future Directions. As the Army moves toward the Objective Force and the troops are more dispersed on the battlefield, units must be more self-sustaining in terms of all support requirements, including medical needs. Evacuation of casualties may be prolonged, requiring the need for medical care through a progression of post-traumatic states over a period of days. A scenario can be envisioned, for example, in which the casualty first needs enhanced hemostasis and then needs treatment for hypothermic and acidotic coagulopathy. Next, the patient may progress to a hyperfibrinolytic state, a state of inappropriate intravascular activation of the coagulation system, or a septic state. Superimposed upon these conditions may be ischemia-reperfusion injury due to resuscitation or a massive inflammatory response. Other confounding factors on the future battlefield that may impact coagulation function include the use of artificial oxygen carriers (blood substitutes), immune modulators, performance enhancers, or other such drugs. The ability to manipulate the hemostatic mechanisms and the way they interact with other systems will therefore be critical to optimize the potential for recovery and survival of the casualty.

Before we can manipulate the hemostatic mechanism, however, we must first understand how the coagulation system responds to trauma and how it impinges upon other systems such as the immune system. In essence, we must better understand the integrated physiological responses to traumatic injury to guide us in the subsequent development of lifesaving strategies. For example, it is currently known that thrombin generation (which is necessary for coagulation) activates the inflammatory system, which can lead to detrimental tissue effects subsequent to hyperinflammation. Modulation of this activation at the level of the endothelial cell may thus allow us to increase coagulation function while decreasing inflammation. As another example, there is very little information currently available about how physiological states that soldiers routinely experience (for example, dehydration, sleep deprivation, cold stress, heat stress, acute exercise, or combinations of these) affect the coagulative response to subsequent trauma. Although no data currently exist that specifically address the effects of these physiological factors on trauma responses, related studies of elite athletes suggest that such stressors may have a profound effect on trauma-related hemorrhage and we see an urgent need to expand on this knowledge base. Finally, when tissue responses to the ischemia produced by blood loss are more fully understood, it may also be possible in the future to supplement hemostatic mechanisms by simultaneously manipulating such responses either through conventional pharmacological means or by new techniques such as gene therapy. Thus, the need for more basic research to understand the physiological mechanisms underlying the response to trauma exists and will continue to grow. The HRP will move to fill this need by performing both clinical and laboratory research to understand the interactions between physiological systems and their response to trauma. Further understanding of the basic mechanisms underlying coagulation and subsequent physiological responses will provide future solutions that not only reduce bleeding in the short-term but will also decrease long-term detrimental effects. Our vision is to continuously improve upon hemostatic tools so that the killed in action rate in future battles will progressively decrease.

References

1. Bellamy RF. The causes of death in conventional land warfare: Implications for combat casualty care research. *Mil Med.* 1984;149:55-62.

2. Sauaia A, Moore FA, et al. Epidemiology of trauma deaths: A reassessment. *J Trauma.* 1995; 38:185-193.

3. Zajtchuk R, Sullivan GR. Battlefield trauma care: Focus on advanced technology. *Mil Med.* 1995; 160:1-7.

4. Melsom MA, Farrar MD, Volkers RC. Battle causalities. *Ann R Coll Surg Engl.* 1975; 56:289-303.

5. Dice WH. The role of military emergency physicians in an assault operation in Panama. *Ann Emerg Med.* 1991; 20:1336-1340.

6. Calkins D, Snow C, Costello M, Bentley TB. Evaluation of possible battlefield tourniquet systems for the far-forward setting. *Mil Med.* 2000; 165:379-384.

7. Pusateri AE, Modrow HE, Harris RA, Holcomb JB, Hess JR, Mosebar RH, et al. Advanced hemostatic dressing development program: Animal model selection criteria and results of a study of nine hemostatic dressings in a model of severe large venous hemorrhage and hepatic injury in swine. *J Trauma.* 2003. In press.

8. Pusateri AE, McCarthy SJ, Gregory KW, et al. Effect of a chitosan-based hemostatic dressing on blood loss and survival in a model of severe venous hemorrhage and hepatic injury in swine. *J Trauma.* 2003; 54:177-182.

9. Sondeen JL, Pusateri AE, Coppes VG, Gaddy CE, Holcomb JB. Comparison of 10 different hemostatic dressings in an aortic injury. *J Trauma.* 003; 54:280-285.

10. Holcomb JB, McClain JM, Pusateri AE, Beall D, et al. Fibrin sealant foam sprayed directly on liver injuries decreases blood loss in resuscitated rats. *J Trauma.* 2000; 49:246-50.

11. Porte RJ, Leebeek FWG. Pharmacological strategies to decrease transfusion requirements in patients undergoing surgery. *Drugs.* 2002; 62:2193-2211.

12. Hedner U. Recombinant factor VIIa (Novoseven®) as a hemostatic agent. *Semin Hematol.* 2001; 38 (suppl 12):43-47.

13. Martinowitz U, Kenet G, Lubetski A, Luboshitz J, Segal E. Possible role of rFVIIa in the control of hemorrhage associated with massive trauma. *Can J Anesth.* 2002; 49:S15-S20.

14. Schreiber MA, Holcomb JB, Hedner U, et al. The effect of recombinant factor VIIa on noncoagulopathic pigs with grade V liver injuries. Submitted.

15. Pusateri AE, Ryan KL, Uscilowicz JM, et al. Effects of increasing doses of activated rFVIIa on hemostasis, coagulation and platelet function in swine. Submitted.

16. Sondeen JL, Pusateri AE, Hedner U, Yantis LD, Holcomb JB. Recombinant Factor VIIa strengthens the clot in uncontrolled aortic hemorrhage. Submitted.

17. Lynn M, Jerokhimov I, Jewelewicz D, et al. Early use of recombinant factor VIIa improves mean arterial pressure and may potentially decrease mortality in experimental hemorrhagic shock: a pilot study. *J Trauma.* 2002; 52:703-707.

18. Martinowitz U, Holcomb JB, Pusateri AE, Stein M, et al. Intravenous rFVIIa administered for hemorrhage control in hypothermic coagulopathic swine with grade V liver injuries. *J Trauma* 2001; 50:721-729.

19. Schreiber MA, Holcomb JB, Hedner U, Brundage SI, Macaitis JM, Hoots K. The effect of recombinant factor VIIa on coagulopathic pigs with grade V liver injuries. *J Trauma.* 2002; 53:252-259.

20. Giles AR, Mann KG, Nesheim ME. A combination of factor Xa and phosphatidylcholine-phosphatidylserine vesicles bypasses factor VIII in vivo. *Br J Haematol.* 1988; 69:491-497.

21. Vaezy S, Martin R, Crum L. High intensity focused ultrasound: A method of hemostasis. *Echocardiography.* 2001; 18:309-316.

22. Noble ML, Vaezy S, Heshavarzi A, Paun M, Prokop AF, et al. Spleen hemostasis using high-intensity ultrasound: survival and healing. *J Trauma.* 2003; 53:1115-1120.

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Development of Hemostatic Dressings for Use in Military Operations

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Introduction

On the battlefield, hemorrhage from wounds remains the leading cause of mortality, accounting for 50% of all deaths.[1] Hemorrhage is also the second leading cause of mortality among injured civilians, accounting for 39% of civilian trauma deaths.[2-4] The primary field-ready methods for control of hemorrhage – tourniquets, direct pressure, bandages, and clamping – have not changed greatly in several centuries.[5] These interventions, even in the hands of experts, are not always effective.[6] In Vietnam, 50% of combat deaths resulted from wounds with uncontrollable bleeding. Of these wounds, about 11% were inflicted in sites accessible for first aid treatment without need for surgical intervention.[1,7] More effective hemostatic methods could have been prevented up to one third of the deaths from exsanguination during the Vietnam War.[1,8] This background information strongly illustrates the need to develop a better hemostatic method to improve the immediate care and survival of casualties in the field.

For the past 9 years, the U.S. Army has worked closely with the American Red Cross (ARC) to develop a field-ready hemostatic dressing that can effectively stop arterial bleeding from major wounds. The ARC has an active program to develop fibrin sealant (FS) hemostatic agents, the focus of one of our research projects. The organization also controls 50% of collected human plasma, the current source of fibrinogen and thrombin proteins, which are the main components of FS dressing. This article briefly reviews the history and development of FS dressing as well as other hemostatic products (for example, chitosan dressing) and the important role that the U.S. Army Institute of Surgical Research (ISR) has played in developing these products.

Historical Background

The first experiments to control bleeding with fibrin date back to 1909, when Bergel reported the hemostatic properties of fibrin powder in the operative field.[9] The first attempt to make a form of dry fibrin hemostatic product for use by trauma surgeons was during World War I, when Grey and Harvey produced pre-polymerized fibrin tampons and thin plaques to control bleeding in parenchymal organs.[10,11] Although these materials are passive hemostatic agents, incapable of polymerizing and cross-linking directly with the tissue, they worked relatively well. The combination of fibrinogen and thrombin was first used in 1944 by Cronkite et al and Tedrick et al for anchoring skin grafts, but because of poor adhesion, it was not widely accepted.[12,13] During World War II, fibrin glue, fibrin sheet foam, and fibrin powder were mass-produced from pooled plasma, but were withdrawn in 1946 because they transmitted hepatitis. Subsequently, in 1977, all pooled human plasma fibrinogen products, including a commercial liquid FS preparation that was licensed in Europe, were recalled by the Food and Drug Administration (FDA) because of the high risk of hepatitis transmission. Today, advances in viral removal (nanofilteration) and inactivation technologies (solvent-detergent, pasteurization, and dry heat treatment), combined with sophisticated donor screening, have reduced the risks of viral transmission from plasma products to extremely low levels. This decreased risk, coupled with strong clinical interest, has led to a resurgence in the development of FS products in the U.S.

Fibrin Sealant

The FS is composed of purified, virally inactivated human fibrinogen and human thrombin that combine to form a fibrin clot. The FS may be used for control of bleeding, tissue gluing, and as a delivery vehicle for drugs and biologics. The hemostatic and adhesive properties of FS are important for certain types of surgical procedures and appear to be useful in treating severe trauma injuries.[14]

The hemostatic function of FS mimics the final stages of the blood coagulation cascade. Once the protein components are dissolved in a fluid (for example, saline or blood), the enzymatic activity of thrombin converts fibrinogen to fibrin monomers by

cleaving small peptides (fibrinopeptides A and B) from the molecules. The fibrin monomers rapidly assemble into fibrils and fiber strands, thereby forming a 3-dimensional gel network. Thrombin also converts inactive factor XIII (FXIII), present with fibrinogen, into its active form (FXIIIa) in the presence of calcium chloride. The FXIIIa transforms the soluble fibrin gel into a dense, insoluble fibrin clot at the bleeding site.[15,16] The fibrin clot binds to the tissue by different modes (covalent, noncovalent, and mechanical bonding) and physically stops the bleeding. In contrast to passive hemostatic agents (collagen, cellulose, etc) that promote the patient's own blood clotting mechanism, FS coagulates independently from patient blood and is therefore useful even in patients with severe coagulopathy. The product is ideally suited for treating traumatic and surgical bleeding in hemophiliac patients.[17]

Liquid FS

Commercial liquid preparations of FS are highly effective when they are used in a conventional setting (for example, a pre-scheduled operation); however, they do suffer from several limitations if they are considered for trauma application. For example, liquid FS is difficult and time-consuming to prepare because it involves hydrating two lyophilized products, which requires warming and prolonged agitation. The liquid mixture can successfully control a majority of surgical oozing that is of low volume and pressure, but it cannot treat high-volume venous or high-pressure arterial hemorrhages, as it is diluted and washed away by the high-volume of blood flowing out of lacerated large vessels. In order to address these limitations and broaden the spectrum of the injuries that can be treated with FS, a new physical form of FS in the shape of a dry dressing was developed.

Dry Fibrin Sealant Dressing (DFSD)

The DFSD technology was developed for both field medical (pre-hospital) and conventional surgical applications. The dressings are simple to use and designed for self-application, buddy-application or administration by an emergency medical technician. The victim or caregiver only needs to open the packaging, remove the product, and press the dressing onto the bleeding wound for approximately 2 to 3 minutes to treat superficial or extremity injuries that may result in significant blood loss. The DFSD may also be used in a hospital setting to control severe parenchymal hemorrhages that either cannot be controlled by conventional methods or require a meticulous operating procedure with the risk of prolonged ischemic time and organ failure.

Early Development of DFSD

The development of DFSD involved several formulation and structural design changes before the final product was ready for manufacturing and distribution. The first prototype of DFSD applicable to trauma surgery was developed at the Letterman Army Institute of Research in the early 1990s.[18] The aim was to deliver a large amount of fibrinogen and thrombin to a wound, producing a fibrin clot with greater density and strength than forms naturally, binding tightly to underlying tissues. The first in vivo test of the prototype dressing, which was composed of powdered fibrinogen and thrombin spread on gauze, was performed at the Letterman Army Institute of Research in 1993. The results, published in 1995, convincingly demonstrated that application of DFSD on an arterial laceration (pig femoral artery) could minimize the bleeding and maintain arterial blood pressure at a normal level.[14]

The original ARC dressing (1st generation) produced with advice from the Letterman team in 1995 had a similar composition (a mixture of dry powdered fibrinogen and thrombin was pressed on a silicon backing material). The backing was intended for handling and application of DFSD onto the wound until hemostasis was achieved, after which it would be removed, leaving behind only the reabsorbable fibrin clot. This prototype was tested in vivo in two severe hemorrhage models including a defined femoral arteriotomy and a complex ballistic injury in the extremity of large animals.[19,20] The dressings produced hemostasis after application and reduced the overall bleeding by 83% (123 ± 48 mL) and 77% (124 ± 64 mL) in both conditions, as compared to the control treatments (standard gauze or placebo dressing) with blood loss of 734 ± 134 mL and 377 ± 64 mL, respectively. The mean arterial pressure was at least 25 mmHg higher in the dressings treated animals than those in the controls. Hemostasis was achieved in the arteriotomy model without compromising the arterial blood flow to the hind leg of the animals.[19]

The first generation of DFSD was later abandoned because of the instability of the powdered materials, which frequently fell off, and the nonabsorbable nature of the cotton and silicon backings. An alternative dressing with absorbable backing was developed (2d generation) that had additional patient benefits. It could be placed in the wound safely and be reabsorbed entirely by the body without the need for removing the backing sheet and disturbing the hemostatic clot. The other necessary change in these dressings was a new method for incorporating fibrinogen and thrombin into the dressing. In the new product, fibrinogen and thrombin were layered (one layer of fibrinogen over one layer of thrombin) on top of an absorbable backing material (Vicryl ™ or Dexon ™ mesh) and freeze-dried into one sheet. The underlying protein layer was attached to the supporting material by the addition of a thin layer of sucrose that embedded the absorbable mesh. However, the ex vivo laboratory testing, which measured the

adhesiveness of the dressing to vascular tissue, revealed very poor adhesive strength of these dressings regardless of which component was the top layer.[21] The reason appeared to be that as soon as saline was added to dissolve the dressing, a thin layer of fibrin clot was formed at the interface of the two proteins and prevented the proper mixing of fibrinogen and thrombin. The result was the formation of a nonhomogeneous clot with low adhesive properties and poor hemostatic efficacy. The structural design of the dressings therefore had to be modified.

In the new design (3d generation), the thrombin layer was placed between two layers of fibrinogen in a "sandwich" configuration. The new design allowed better mixing of active components and complete polymerization of the fibrinogen into a uniform fibrin clot that showed superior adhesive properties.[21] The concentration of fibrinogen, which is the critical factor in determining the adhesive strength of the dressing, was optimized (15 mg/cm^2) based on the results of a study using large animals that was conducted at William Beaumont Army Medical Center, in El Paso, TX.[22]

The final laboratory-produced DFSD (Figure 1) was tested extensively in a variety of innovative hemorrhage models developed by ISR scientists to determine the efficacy and potential benefit in military operations. For example, a model of severe liver injury was developed in large animals (pigs) using a custom-designed clamp with two 4.5 cm sharpened blades that lacerated major hepatic veins and liver tissue in a manner similar to a gunshot injury.[23] Hemorrhage in this model is so severe that it often results in exsanguination of the animal if not treated (Figure 2). The same injury was also produced in animals in which their natural blood clotting capacity was severely diminished by blood dilution and hypothermia (coagulopathy syndrome), similar to conditions that develop in trauma patients or in battlefield casualties. In these circumstances, the bleeding is more persistent, harder to control, and more likely to be fatal if not treated promptly. The DFSD controlled these life-threatening hemorrhages in both models and offered a simple and effective method of hemostasis that was superior to standard care (gauze packing) practiced in hospitals.[24] The long-term effects of the dressings were also evaluated in specialized urological operations performed in large animals. For example, application of DFSD dressing on the prostatic bed following prostatectomy (removal of the prostate gland) reduced blood loss by 25 to 30% and shortened the operation time to half that required for other groups.[25] Similarly, when DFSD was used to treat bleeding from the kidney after a partial nephrectomy operation or a stab wound injury, the hemorrhage was stopped more rapidly (less blood loss) and the surgery was completed in a shorter time resulting in less ischemic injury compared with standard surgical techniques.[26,27] The secondary bleeding and leakage of urine from the injured portion of the kidney were also prevented during the 6-week postoperative recovery period, during which time the organ healed properly. These long-term studies were conducted at Brooke Army Medical Center in San Antonio, TX.

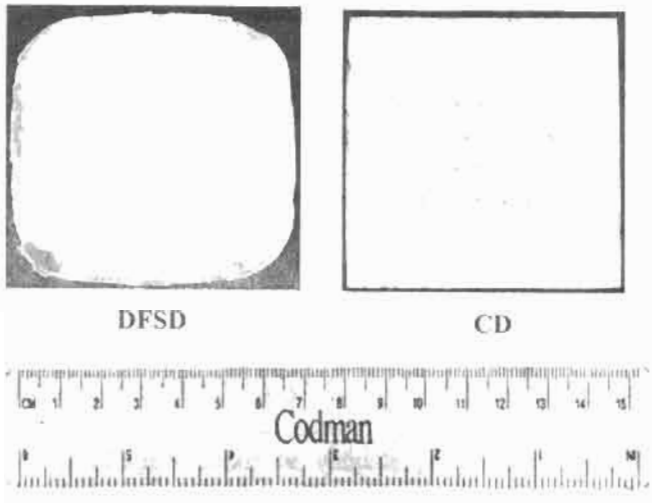

Fig 1. Photographs of the hemostatic dressings that were successfully tested and currently distributed in far-forward military operation overseas. ***DFSD****: The dry FS dressing (final production) that is composed of human fibrinogen and thrombin with an absorbable backing material and made by the ARC.* ***CD:*** *The chitosan dressing derived from a natural polysaccharide known as chitin found abundantly in shellfish. This dressing is manufactured by HemCon, Inc.*

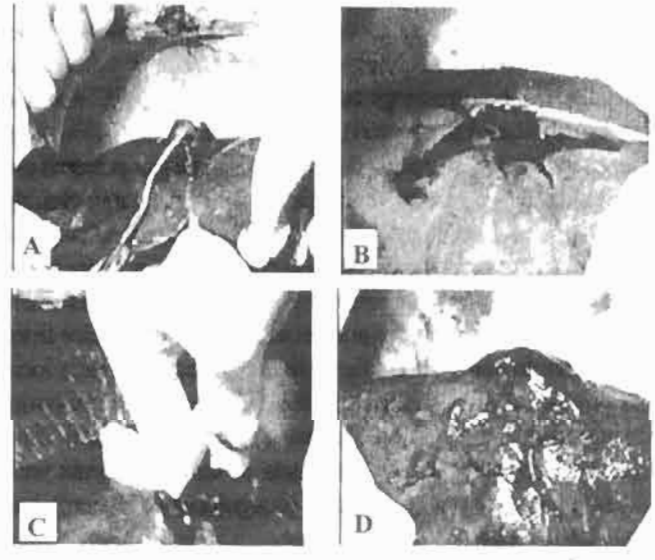

Fig 2. Photographs of the severe liver injury and profuse venous bleeding model in swine. ***A****. The liver tissue and underlying major veins of the two medial lobes are lacerated twice using a custom-designed clamp with sharp blades (X shape).* ***B****. The result appears as a large stellate wound (approx 10 X 8 X 4 cm) similar to a gunshot injury.* ***C****. The wound is packed with three dressings and held for 4 min (or shorter if hemostasis is achieved), after which the animal is closed, resuscitated and monitored for 1 hour.* ***D****. Treatment with DFSD produced hemostasis during the 4-min compression period and bleeding is completely stopped.*

Selection of Best Hemostatic Dressing for Military Use

In addition to DFSD, ISR investigated a number of other hemostatic dressing products with potential benefit to soldier care. Some of these agents were already approved (for example, Surgicel, Avitene) and some were under development to be licensed by the FDA and used in a variety of clinical settings. The U.S. Army wants to select the most effective hemostatic dressing that meet the far-forward needs of military use. The ideal hemostatic dressing, as defined by the U.S. Special Operation Command, should meet the following conditions:

- Able to stop large-vessel arterial and venous bleedings within 2 minutes after application on the wound.
- Ready to use; no mixing or special preparation.
- Simple to apply by the wounded personnel, his buddy, or medics without any training.
- Stable at room temperature for at least 2 years and in extreme temperatures (between 40 and -10°C) for several weeks or longer.
- Safe to use and pose no risk of bacteria or viral transmission.

Companies and organizations were solicited to submit their products for vigorous testing to see which one would meet the above criteria. A total of nine hemostatic dressings, including two FS dressings (one made by the ARC and the other by Nycomed in Austria), were submitted for evaluation. Except for the FS dressings, the remaining dressings tested are considered to be passive hemostats, promoting the clotting mechanisms of the patient's own blood as a means to stop the bleeding. In general, passive hemostats act by enhancing platelet aggregation, accelerating intrinsic and extrinsic pathways of clot formation, and protecting the blood clot from degradation. In some cases, they were claimed to cause a local vasoconstriction (for example, the Marine algae polymer dressing) and thereby reduce bleeding from the vessels. The reduced efficacy of passive hemostats in trauma patients with decreased blood clotting capacity (coagulopathic patient) has been acknowledged.

The dressing candidates were subjected to two severe hemorrhage tests in large animals at the ISR. In the first study, the dressings were applied to grade V liver injury in swine (described above), which represents high-volume venous bleeding.[28] The efficacy of each dressing was compared individually with a control treatment in which cotton gauze was used to stop the hemorrhage. The ARC FS was found to be the only product that significantly increased hemostasis and reduced bleeding when compared with control treatments. In the second study, the dressings were tested in an even more challenging model that involved severe high-pressure arterial bleeding that produced 100% fatality within 10 minutes after injury in untreated animals.[29] The hemorrhage was produced by making a 4.4 mm diameter hole in the abdominal aorta of the pig, which was allowed to bleed freely for 6 seconds. While bleeding continued, dressings were placed through a pool of blood over the injury site and pressure held for 4 minutes. Hemostasis was determined following removal of manual compression. For controls, either the Army standard gauze dressing was used (negative control) or the vessel was clamped and properly sutured (positive control). The only dressing that effectively sealed the vascular injury and prevented further blood loss and the death of the animals was found to be the FS dressing made by the ARC (Figure 3). The efficacy of this dressing during a 1-hour observation period (short-term) was equal to that of the standard suturing technique. Animals treated with other hemostatic dressings exsanguinated during the observation period.

Fig 3. Photograph of the aortic injury and fatal arterial hemorrhage model in swine. A 4.4 mm hole in the abdominal aorta is sealed and hemorrhage is prevented with the use of a DFSD. The dressing completely adheres to the blood vessel and surrounding tissue (From 29; Reprinted with permission from Lipincott Williams & Wilkins).

Manufacturing of DFSD

Although none of the nine dressings tested met all of the desired criteria for military use, the ARC DFSD dressing met the more important requirements including those for high efficacy, ready-to-use, stability at room temperature, and biological safety of the product. The promising outcomes of the animal studies, along with a well-planned proposal submitted by the ARC and a manufacturing facility (CSL Bioplasma, Broadmeadows, Victoria, Australia) prompted substantial financial support by the U.S. Army Medical Research and Materiel Command to advance the production of this dressing from the laboratory bench to a large-scaled manufacturing facility in 2001.

One of the shortcomings of the ARC DFSD, noticed during testing, was the fragile nature of the fibrinogen sheets, which break down easily and slough off when the dressings were handled. One reason for this problem was the incorporation of thrombin as a thin layer between the two fibrinogen sheets in a sandwich configuration. Because of the differences in protein and buffer composition between fibrinogen and thrombin, the crystallized thrombin layer acted as a weak interface between the dry fibrinogen sheets and reduced the firm attachment of the fibrinogen layers. As a result, the upper layer of fibrinogen was frequently delaminated and easily flicked off during handling or rough transportation. To minimize this problem, two modifications were made at the manufacturing level. First, the dressings were made, stored, and transported in rigid plastic containers so that they would be protected from some inevitable hits and damage during transportation. Second, the design for incorporating thrombin into the dressing was changed. This modification required further experimentation to prove the equivalency of the scaled-up production dressing with the laboratory-made dressings that were tested earlier.

In the newly-designed dressing, thrombin was placed as minute aliquots (~100 dots, 1 cm apart), spread evenly over the first layer of fibrinogen and covered with the second layer of the fibrinogen. This "polka dot" arrangement allowed better attachment of fibrinogen layers, particularly on the area void of thrombin, and easier way toward total automation of dressing production and robotic application of the components. Initial in vitro tests performed at the manufacturing facility did not show any significant difference between the new dressing design and the prototype in which thrombin was sprayed as a continuous layer. This result, however, had to be confirmed in a challenging in vivo study to ensure equal efficacy of the dressings.

The equivalency study was performed at the ISR and the dressings (prototype vs first polka dot design) were tested in the liver hemorrhage model (described earlier) in normal swine.[30] Hemostasis achieved with each dressing was compared with that of a control group in which the liver injury was treated with standard gauze. Although the polka dot design reduced the hemorrhaging, it appeared to be less effective in reducing blood loss (31% larger blood loss than the prototype design), and achievement of hemostasis was not substantially better than with the gauze. This result clearly indicated that, despite the favorable in vitro test results by the manufacturer, the in vivo efficacy of the newly-designed dressings was not equal to the prototype. The lower efficacy of the polka dot design appeared to be due to inadequate mixing of thrombin and fibrinogen and therefore incomplete polymerization of fibrinogen across the dressing once it was dissolved in blood.

A new pattern with better distribution of thrombin throughout the dressing seemed to be a logical approach to improve the mixing and consequently the hemostatic function of the product. This was achieved by dividing the same amount of thrombin into smaller aliquots and evenly distributing them between the fibrinogen layers. This design retained the advantage of preventing the delamination of fibrinogen layers but allowed more direct contact of the thrombin with fibrinogen and better mixing upon dissolution. The new polka dot designed dressings, along with the prototypes, were subjected to the same in vivo testing by the ISR staff, using the severe liver hemorrhage model in swine.[30] The results showed significant improvement in hemostatic function of the new product. The efficacy of scaled-up production was equal to the prototype dressings produced in the laboratory setting. Last year, this dressing received preliminary approval by FDA in the military arena for treating external injuries and is currently distributed among Special Operations Forces under an Investigational New Drug protocol.

Chitosan Hemostatic Dressing

Recently, a new hemostatic dressing has been developed by the Oregon Medical Laser Center with potential utility to control severe extremity hemorrhages (Figure 1). The dressings are made of chitosan, a derivative of a natural polysaccharide known as chitin found abundantly in shellfish (for example, shrimp). Various forms of chitin and chitosan have been shown to enhance hemostasis in experimental studies. The chitosan dressings offer several advantages over DFSD with regard to the durability and ease of application, particularly in the far-forward military arena. Application of these dressings over wounds does not require any special precautions or use of nonadhesive gloves, which may be necessary for DFSD. They also seem to maintain their adhesive function even if they become wet before being placed on the wounds. Because of their chemical structure (very similar to cellulose), these dressings are more stable and tolerant to prolonged exposure to extreme temperatures (-50° to +140°F). The chitosan dressing was not available when the hemostatic efficacies of the other nine dry dressings were compared; however, the encouraging preliminary data provided by the manufacturer prompted evaluation of a chitosan dressing (prototype) in the standard liver hemorrhage model in swine.[31] The results of this study at the ISR showed that chitosan dressing treatment could significantly reduce blood loss, mortality rate, and enhance hemostasis, when compared with a control treatment (cotton gauze).

More recently, the manufacturing company (HemCon, Inc) received FDA approval for the use of the chitosan dressing to treat external bleeding. This led to large-scale manufacturing and production of hundreds of dressings ready for shipment to the battlefield. The limited in vitro and in vivo tests carried out

by the company showed no significant differences between the scaled-up production and the prototype versions previously tested successfully at ISR. However, the experience with the DFSD development obligated ISR and the Army to thoroughly test the product before recommending its shipment and distribution among the troops.

Samples of production dressings were subjected to a standardized model of liver hemorrhage in swine. Data were compared with results obtained using the prototype chitosan dressing under identical study conditions. The production version of the dressing did not improve initial hemostasis, nor change the overall bleeding or survival rate, compared with gauze used as control dressings. These results clearly indicated that the efficacy of the mass-produced dressings was inferior to the prototype version and not suitable for release in the military arena.

This conclusion led to rapid improvements in the manufacturing process of the chitosan dressings by HemCon, Inc and production of a new version that was more flexible and absorbant with better adhesive properties than the previous product. The new dressings were expeditiously (in 1 day) tested in the standard hemorrhage model with participation of nearly the entire ISR staff. Once the hemostatic efficacy of these dressings was confirmed in the large animal study, shipment of the final product was recommended for possible treatment of combat casualties in Iraq and Afghanistan.

Conclusion

The results of nearly a decade of laboratory and animal research are the development and production of two highly effective hemostatic dressings with potentially lifesaving properties. These dressings are presently distributed among the soldiers in far-forward military operations overseas. Each product has its own unique advantages and may be more suitable for use in special circumstances. The chitosan dressings are more stable, durable, easy to use, and less expensive. They are more likely to be utilized in the first aid stage for temporary control of bleeding. On the other hand, DFSD's are more flexible (after contact with blood) and better able to conform and adhere to a complex injury, with proven efficacy against the most aggressive and life-threatening hemorrhages. The required careful application process of the DFSD is a potential limiting factor for widespread use of this product.

This report summarizes the important role that the U.S. Army has played in the development of hemostatic products relevant to military needs and treatment of combat casualties. The invention and employment of challenging hemorrhage models in large animals has been essential in identifying the most promising hemostatic dressings and guiding these products through manufacturing and optimization processes to the extent that they are now available for use by our Armed Forces.

References

1. Bellamy RF. Causes of death in conventional warfare. *Mil Med.* 1984;149:55-62.

2. Acosta JA, Yang JC, Winchell RJ, et al. Lethal injuries and time to death in a level one trauma center. *J Am Coll Surg.* 1998;186:528-533.

3. Sauaia A, Moore FA, Moore EE, et al. Epidemiology of trauma deaths: a reassessment. *J Trauma.* 1995;38:185-193.

4. Shackford SR, Mackersie RC, Holbrook TL, et al. The epidemiology of traumatic death: a population-based analysis. *Arch Surg.*1993;128:571-575.

5. Zimmerman L, Veith I. *Great ideas in the history of surgery.* San Francisco, CA: Norman publishing;1993.

6. Rocko JM, Tischler C, Swan KG. Exsanguination in public: A preventable death. *J Trauma.* 1982;22:635.

7. Bzik KD, Bellamy RF. A note on combat casualty statistics. *Mil Med.* 1984; 149:229-30.

8. Zajtzchuk R, Sullivan GR. Battlefield trauma: focus on advanced technology. *Mil Med.* 1995;160:1-7.

9. Bergel S. Uber wirkungen des fibrins. *Dtsch Med Wochenschr.* 1909; 35: 633.

10. Grey EG. Fibrin as a hemostatic in cerebral surgery. *Surg Gynecol Obstet.* 1915;21: 452.

11. Harvey SC. The use of fibrin papers and forms in surgery. *Boston Med Surg J.* 1916;174:658.

12. Cronkite EP, Lozner EL, Deaver JM. Use of thrombin and fibrinogen in skin grafting. *JAMA.* 1944;124:976.

13. Tedrick RT, Warner ED. Fibrin fixation of skin transplants. *Surgery.* 1944;15:90.

14. Larson MJ, Bowersox JC, Lim RC, Hess JR. Efficacy of a fibrin hemostatic bandage in controlling hemorrhage from experimental arterial injuries. *Arch Surg.* 1995;130:420-422.

15. Hermans J, McDonagh J. Fibrin structure and interactions. *Semin Thromb Hemostas.* 1982;8:11-24.

16. Hantgan RR, Francis CW, Marder VJ. Fibrinogen structure and physiology. In: Coman RW, Hirsh J, Marder VJ, Salzman EW, ed. *Hemostasis and Thrombosis: Basic Principles and Clinical Practice. 3 ed.* Philadelphia, PA: Lippincott Co; 1994 p 277-300.

17. Tock B, Drohan W, Hess J, Pusateri A, Holcomb J, MacPhee M. Hemophilia and advanced FS technologies. *Haemophilia.* 1998;4:449-55.

18. Holcomb JB, Pusateri AE, Hess JR, et al. Implications of new dry FS technology for trauma surgery. *Surg Clin of North Am.* 1997;77:943-52.

19. Jackson M, Friedman SA, Carter AJ. Hemostatic efficacy of a FS-based topical agent in a femoral artery injury model: a randomized, blinded, placebo-controlled study. *J Vasc Surg.* 1997; 25:274-280.

20. Holcomb J, MacPhee M, Hetz S, et al. Efficacy of a dry FS dressing for hemorrhage control after ballistic injury. *Arch Surg*. 1998;133:32-35.

21. ARC final report: Advanced field-ready fibrin-based hemostatic devices. Award No: DAMD 17-99-2-9002. December .1999; p 12-15.

22. Pusateri AE, Holcomb JB, Harris RA,et al. Effect of fibrin bandage fibrinogen concentration on blood loss after grade V liver injury in swine. *Mil Med*. 2001;166:217-22.

23. Holcomb JB, Pusateri AE, Harris RA, et al. Effect of dry fibrin sealant dressings on blood loss in grade V liver injuries in resuscitated swine. *J Trauma*. 1999; 46:49-57.

24. Holcomb JB, Pusateri AE, Harris RA, Reid TJ, et al. Dry FS dressings reduce blood loss, resuscitation volume, and improve survival in hypothermic coagulopathic swine with grade V liver injuries. *J Trauma*. 1999; 47:233-242.

25. Cornum R, Bell J, Gresham V, Brinkley W, Beall D, MacPhee M. Intraoperative use of the absorbable fibrin adhesive bandage: long-term effects. *J Urol*. 1999; 162:1817-1820.

26. Cornum RL, Morey AF, Harris R, Gresham V, et al. Does the absorbable fibrin adhesive bandage facilitate partial nephrectomy? *J Urol*. 2000;164:864-867.

27. Morey AF, Anema JG, Harris R, Gresham V, et al. Treatment of grade 4 renal stab wounds with absorbable fibrin adhesive bandage in a porcine model. *J Urol*. 2001; 165:955-958.

28. Pusateri AE, Modrow HE, Harris RA, Holcomb JB, Hess JR, et al. Advanced hemostatic dressing development program: animal model selection criteria and results of a study of nine hemostatic dressings in a model of severe large venous hemorrhage and hepatic injury in swine. *J Trauma*. 2003. In press.

29. Sondeen JL, Pusateri AE, Coppes VG, Gaddy CE, Holcomb JB. Comparison of 10 different hemostatic dressing in an aortic injury. *J Trauma*. 2003;54:280-85.

30. Pusateri AE, Kheirabadi BS, Modrow HE, et al. Use of a model of severe hepatic injury and venous bleeding to evaluate efficacy of the dry FS dressing and improve the manufacturing process. *J Biomed Mater Res*. Submitted.

31. Pusateri AE, McCarthy SJ, Gregory KW, et al. Effect of a Chitosan-based hemostatic dressing on blood loss and survival in a model of severe venous hemorrhage and hepatic injury in swine. *J Trauma*. 2003;54:177-182.

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Fluid Resuscitation Research for the Treatment of Significant Hemorrhage

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Hemorrhage is the primary cause of death on the battlefield in conventional warfare. Future combat strategies focused around the Objective Force Warrior and greater dispersal of troops, suggest the likelihood of longer evacuation times for combat casualties. As a consequence, fluid resuscitation research at the Institute of Surgical Research (ISR) focuses on investigating limited- or small-volume fluid resuscitation strategies for use in far-forward areas. This includes permissive hypotension to treat severe hemorrhage on the battlefield to improve survival and to reduce or prevent early and late deleterious sequelae. As there are little data available on the consequences of permissive hypotension coupled with longer evacuation times for the military, these studies have important implications in the development of optimal fluid resuscitation strategies for stabilization of the combat casualty.

Introduction

Acute hemorrhage accounts for about 50% of battlefield deaths in conventional warfare, and for 30% of evacuated casualties who die from wounds.[1] Overall, these data estimate that 65%-80% of casualties may require some amount of fluid. In addition, lessons learned in the Falkland Islands War, in past Arab-Israeli conflicts and from military skirmishes in Northern India, confirm that prompt resuscitation improves survival.[2,3] In a recent consensus conference, Butler et al recommended fluid resuscitation for any casualty with a change in mental status or who was unconscious, a condition that would suggest a systolic blood pressure less than 50 mmHg.[4]

It is well recognized that limitations exist in providing fluid resuscitation in far-forward combat environments. Weight and cube limitations restrict the availability of large volumes of crystalloid resuscitation fluids for far-forward use, and there can be significant time delays and failure rates in obtaining intravenous access of peripheral veins. In addition, the combat medic has limited training, and evacuation times to forward surgical facilities will likely increase in the future. However, evidence from studies in experimental animals suggests that interventions to re-establish homeostasis may need to be initiated within 30 minutes after injury to assure survival, further challenging attempts to improve survival on the battlefield.[5]

By all indications and as learned in Somalia, future combat scenarios under the Objective Force, with a greater dispersal of troops, may commonly see casualty evacuation times exceeding 24 hours, particularly if evacuation is from urban environments.[6] Taken together, the implication is that at a minimum, several hours may pass before any surgical intervention to treat the injured soldier is possible. Accordingly, special operations forces operate under the assumption that evacuation of casualties may approach 72 hours. As indicated by Bellamy, mortality increased from 20% to 32% when evacuation times of casualties were increased from within 1 hour to 24 hours, respectively.[1] His data also indicate, as expected, that the predicted mortality rate would continue to rise if evacuation times exceeded 24 hours.

Permissive Hypotension

Based on this information, one of the goals of the U.S. Army's Combat Casualty Care program is to develop a strategy to improve field fluid resuscitation for the treatment of significant hemorrhage in combat casualties expecting longer evacuation times and limited availability of resources. The concept of permissive hypotension, or fluid resuscitation to a blood pressure lower than normal, as a far-forward treatment strategy for special operations forces, grew out of a workshop held at the 1998 Special Operations Medical Association meeting but, permissive hypotension was recognized as a reasonable approach in the care of combat casualties in both World Wars I and II.[4,7,8]

Today, traditional fluid resuscitation practices to normalize blood pressure rapidly are being challenged, especially for treating hemorrhagic shock victims with penetrating injuries.[9,10] Even for blunt trauma patients, the wisdom of rapid volume infusion is being questioned.[11] It has been argued that resuscitation to baseline or normal blood pressure can increase bleeding and worsen outcome because of severe hemodilution and disruption of newly forming blood clots. Thus, it is hoped that permissive hypotensive resuscitation can improve outcome, yet avoid these adverse hemostatic effects.[9-13] For example, studies in experimental animals have shown that in the treatment of uncontrolled hemorrhage from a vascular injury, restoring blood pressure to 40 or 60 mmHg resulted in longer survival compared to animals resuscitated to the baseline mean arterial pressure (MAP) of 80 mmHg or animals that received no fluid.[14,15] In addition, providing some fluid before surgical repair of the injury appeared to produce

better results than delaying all fluid until after surgery.[14] Recently, a study in our laboratory showed that fluid resuscitation with lactated Ringer's (LR) to a MAP of 70 mmHg improved hemorrhage-induced vascular hyporeactivity to norepinephrine better than LR resuscitation to baseline MAP during the 4-hour study period.[16] Rats were anesthetized and hemorrhaged to a MAP of 50 mmHg for 60 min. This degree of hemorrhage corresponded to about 19 ± 2 mL/kg body weight. The rats were then resuscitated with different fluids (Table 1) to achieve and maintain a MAP of 70 mmHg and monitored for up to 4 hours or until death. An additional group received LR infusion to return MAP to pre-hemorrhage levels. Resuscitation to baseline MAP with LR resulted in severe hemodilution and deterioration of vascular responsiveness to norepinephrine.[16]

LR[2]	HS-LR[3]	Hespan	Hextend	LR-BL[4]
156 ± 23	100 ± 14*	17 ± 2*	24 ± 5*	399 ± 30*
(Volume infused [mL/kg])				

[1]Data expressed as mean ± SME of n = 7/group

[2]Lactated Ringer's group

[3]5% Hypertonic saline during first hr and LR thereafter

[4]LR resuscitation to baseline MAP

*$P<0.05$ as compared to the LR group

Table 1. Infusion Volume of Each Fluid to Maintain MAP of 70 mmHg After Hemorrhage in Rats[1]

Despite the results observed in the above studies, the adequacy of hypotensive fluid resuscitation has recently been questioned. For example, studies have suggested that hypotensive crystalloid resuscitation to a MAP of 60 to 70 mmHg may be inadequate to prevent metabolic derangements associated with hemorrhagic shock.[17,18] It should be noted, however, that over the last decade of research into hypotensive resuscitation, the majority of studies have only followed animals for a few hours and LR or normal saline has been the primary fluid examined.[19,20] Also, only one study in rats and one in pigs has extended the observations to 72 hours.[14,15] Since not all animals in the hypotensive resuscitation groups survived in some of these studies, further investigation warrants use of different fluids, resuscitation to a higher blood pressure, or resuscitation to better physiologic endpoints in an attempt to improve outcome.

Small Volume Resuscitation

To compensate for the logistical problems of providing enough crystalloid fluids on the battlefield to resuscitate the injured soldier adequately, the U.S. Army initiated studies to investigate the potential efficacy of resuscitation fluids that could be effective in small volumes. This led to an extensive effort to evaluate 7.5% NaCl/6% hypertonic saline dextran-70 (HSD). Results from pre-clinical and clinical studies have shown that HSD could be at least as effective as LR for the treatment of significant hemorrhage, but at 1/10-1/12 the volume of LR.[21-26] The differences in volume requirements of various fluids are illustrated in Figure 1. The premise here is that for treating a 1L blood loss, 3L of LR would be the standard fluid resuscitation strategy. In contrast, only 1L of a colloid solution such as Hextend (illustrated), Hespan or a hemoglobin therapeutic would be required. Combining a colloid with a hypertonic crystalloid further reduces the fluid requirement such that only a 250 mL bag of HSD (illustrated) would be required to provide similar hemodynamic improvement as the 3L bag of LR. The implication of this research strategy on reducing the logistic burden on the battlefield is obvious.

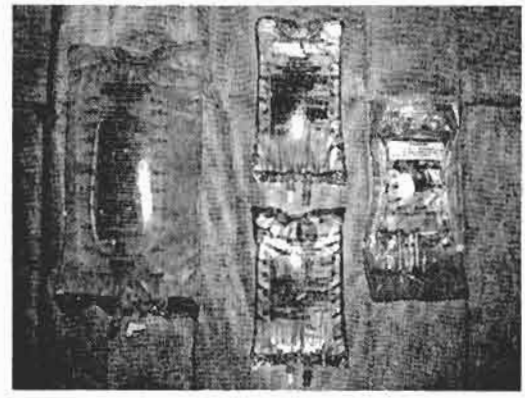

Fig 1. Theoretical fluid volume requirements to achieve equal resuscitation after a 1L blood loss. Pictured are a 3L bag of LR, 2-500 mL bags of Hextend, and a 250 mL bag of HSD.

The treatment of significant hemorrhage requires fluid resuscitation, but it is recognized that the presence of hypotension, environmental and tactical conditions, limited expertise of the medic and/or the presence of mass casualties can lead to significant time delays and failures in gaining access to peripheral veins in the far-forward combat arena. Studies conducted over the past 20 years have renewed interest in the use of intraosseous (IO) infusion as a viable alternate route for the emergency injection of drugs and fluids and the technique was easy to learn by military first responders.[27,28] The U.S. Army, through in-house research activities and outside contracts, has examined IO infusion as an alternative means of infusing resuscitation fluids for the treatment of hemorrhagic hypotension in experimental animals.[29-32] These studies have focused on HSD and have observed that a single dose of HSD induced essentially identical hemodynamic effects through either IO or intravenous infusion.[29-31] In addition, studies suggested that IO infusion of isotonic crystalloids could not resuscitate from hemorrhagic hypotension in a timely manner but IO administration of HSD could be effective.[30-32]

A pilot study was initiated to determine whether HSD, infused through the IO route, could be used in the context of permissive hypotension to resuscitate animals subjected to an uncontrolled hemorrhage.[33] Anesthetized, splenectomized pigs were bled 25 mL/kg (about 37% of estimated blood volume) from the femoral artery over a 30 minutes period. An uncontrolled hemorrhage was induced by pulling the aortotomy wire, and the animal was left undisturbed for 15 minutes. Fluid resuscitation with HSD or LR was initiated through an IO sternal access device until a systolic blood pressure of 70 mmHg was achieved. Pressure was maintained at this level with the appropriate fluid over a 2-hour experimental period. The results of these studies indicated that the volume of HSD required to maintain systolic blood pressure at 70 mmHg was less than 10% of the necessary volume of LR, similar to the data obtained with bolus infusions of HSD.[22,23]

Current Studies

In addition to HSD, hypotensive resuscitation research in our laboratory has expanded to evaluate U.S. Food and Drug Administration-approved fluids or other investigational products. One of our goals is to evaluate whether any of these fluids, when used in hypotensive resuscitation, is superior in improving hemodynamic and metabolic responses to severe hemorrhage. In an ongoing study, anesthetized, splenectomized swine are hemorrhaged 20 mL/kg followed by an additional 8 mL/kg after a 30 minute compensation period. Each hemorrhage period duplicates the blood loss profile of an uncontrolled hemorrhage. Thus, the model mimics an uncontrolled hemorrhage, yet retains the reproducibility of a controlled hemorrhage. Also, this model is lethal if left untreated. Fluid resuscitation is begun 30 minutes after the first hemorrhage. The second hemorrhage is then begun to mimic rebleeding that may occur with resuscitation. Fluid resuscitation is continued as needed to achieve and maintain a systolic blood pressure of 80 mmHg. Animals are monitored for 3 hours after the start of fluid infusion or until death. Figure 2 illustrates preliminary results of LR resuscitation versus no treatment on blood pressure in these animals.[34] Of note, resuscitation with LR to a systolic blood pressure of 80 mmHg resulted in 100% survival from an otherwise lethal hemorrhage, but the volumes required were 3.4 times the shed blood volume, exceeding the typical 3:1 ratio of resuscitation fluid to blood volume loss often used in standard resuscitation. Other fluids investigated in this study include Hespan and Hextend as colloids, 5% hypertonic saline, and a hemoglobin therapeutic as an oxygen carrier. It should be noted that Special Forces medics now carry Hextend.

Preliminary results in Table 2 show the volumes of the different fluids necessary to achieve and maintain a systolic blood pressure of 80 mmHg, and illustrates the potential volume sparing effects of colloids or hypertonic fluids. Similar volume

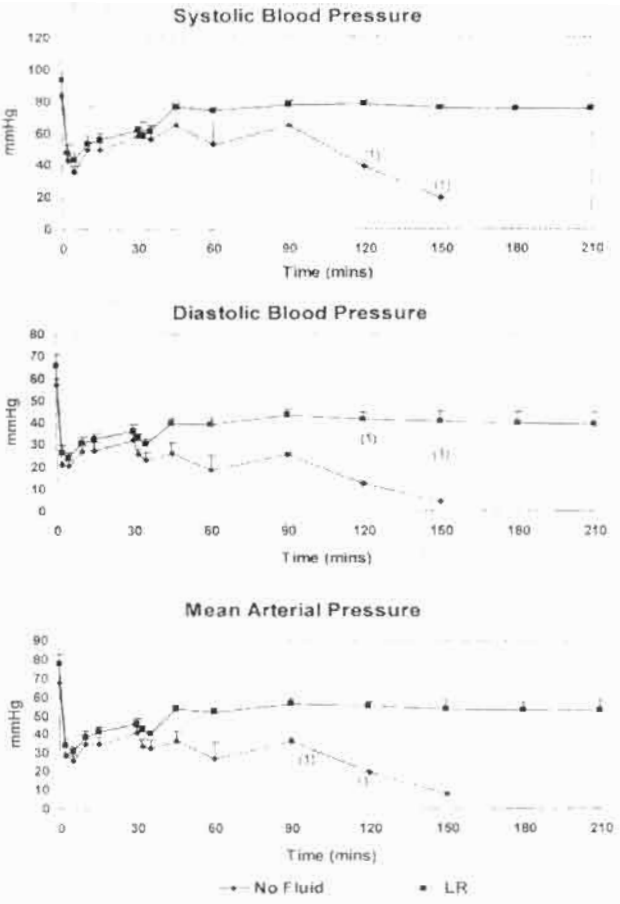

Fig 2. Systolic, diastolic, and MAP in hemorrhaged swine receiving either no treatment or fluid resuscitation with LR.

sparing effects of colloids were observed in Table 1 to maintain MAP at 70 mmHg after hemorrhage in rats as described above.[16] In addition, a parallel study using these fluids is being conducted in conscious pigs to evaluate the effects of permissive hypotension for up to 24 hours. Chronically instrumented, splenectomized pigs are hemorrhaged 37 mL/kg following the same uncontrolled hemorrhage profile described above. When the MAP falls below 40 mmHg, an aortotomy wire is pulled to initiate an uncontrolled hemorrhage and to allow for the evaluation of rebleeding after fluid resuscitation. In this model, fluid resuscitation begins 10 minutes after hemorrhage and continues to achieve and maintain a systolic blood pressure of 80-82 mmHg. After 24 hours, the aortotomy is surgically repaired and animals receive their shed blood back and allowed to recover. Animals are monitored for up to 72 hours after the start of resuscitation or until death to begin to evaluate potential complications (for example, signs of organ failure, as a consequence of prolonged hypotensive resuscitation).

Concluding Remarks

Current studies now investigate fluid resuscitation

No Resuscitation	LR[2]	Hespan	Hextend
0	88.8 ± 17.7	33.2 ± 16.3*	33.6± 9.7*
	(Volume infused [mL/kg])		
5% NaCl	**HSD[3]**	**HBOC[4]**	
16.0 ± 5.9*	10.4 ± 2.8*	29.7± 0.8*	
	(Volume infused [mL/kg])		

[1]Data expressed as mean ± SEM of n = 2-5/group

[2]Lactated Ringer's group

[3]Hypertonic saline dextran

[4]Hemoglobin oxygen carrier fluid

*$P<0.05$ from lactated Ringer's group

Table 2. Infusion Volume of Each Fluid to Maintain Systolic Blood Pressure of 80 mmHg after Hemorrhage in Pigs[1]

practices under the concept of permissive hypotension. However, much remains to be investigated to determine the optimal fluid that can be used in small volumes, yet improve outcomes even in situations where definitive care is delayed for many hours after injury. It is interesting to note that after years of resuscitation of thousands of patients in the treatment of hemorrhage, little reliable evidence exists to suggest how much fluid to give or the clinical end points to guide resuscitation.[35] Although some animal studies seem promising, the long-term effects of permissive hypotension and the lasting superiority of one type of fluid over another are unknown. To this end, other studies at the ISR and discussed elsewhere in this issue, investigate the genetic responses to hemorrhage and resuscitation. Employing state-of-the-art microarray technology, these studies use genomics to evaluate the metabolic consequences of hemorrhage to provide a better understanding of the potential complications of prolonged hypotensive resuscitation. Although evidence suggests that resuscitation to a systolic blood pressure of 80 mmHg may be inadequate to improve cerebral perfusion after head injury, the addition of adjuncts or a "designer" fluid might improve overall tissue perfusion when used with permissive hypotension.[36,37] As noted, to date most fluid resuscitation studies evaluating permissive hypotension have utilized crystalloids such as LR or normal (physiologic) saline. Recently, Burris et al suggested that at least short-term outcome can be improved by resuscitating to a lower blood pressure with a hypertonic saline-hetastarch fluid rather than with LR.[38] Additionally, fluid resuscitation in conjunction with new hemostatic agents, described elsewhere in this Journal issue, is another direction for future research at the ISR.

In summary, earlier research in our laboratory on HSD and IO infusion, as part of the Combat Casualty Care fluid resuscitation task area, illustrates the likelihood that resuscitation fluids required in lower volume, and easier to administer for resuscitating injured soldiers from severe hemorrhage in the far-forward combat environment, can be developed. It is anticipated that successful implementation of hypotensive resuscitation strategies far-forward with the best available fluid or improved treatment strategies will conserve limited resources, decrease rebleeding complications, and improve the likelihood that injured soldiers will reach medical treatment facilities, thereby reducing the number killed in action.

References

1. Bellamy RF. The causes of death in conventional land warfare: Implications for combat casualty care research. *Mil Med.* 1984; 149:55-62.

2. Dubick MA, Kramer GC. Hypertonic saline dextran and intraosseous vascular access for the treatment of hemorrhagic hypotension in the far-forward combat arena. *Annals Acad Med Singapore.* 1997; 26:64-69.

3. Mehrotra M, Mehrotra S. Provisions of trauma resuscitation and anesthesia service in an advance field military hospital in northern India. *Trauma Care J.* 2002;12:18-21.

4. Butler FK, Hagmann JH, Richards DT. Tactical management of urban warfare casualties in special operations. *Mil Med.* 2000; 165(suppl 1):1-48.

5. Nelson AW, Swan H. Hemorrhage: Responses determining survival. *Circ Shock.* 1974;1:273-285.

6. Mabry RL, Holcomb JB, Baker A, et al. U.S. Army Rangers in Somalia: An analysis of combat casualties on an urban battlefield. *J Trauma.* 2000;49:515-529.

7. Cannon WB, Fraser J, Cowell EM. The preventive treatment of wound shock. *JAMA.* 1918; 70:618.

8. Beecher HK. Preparation of battle casualties for Surgery. *Ann Surg* 1945;121:769-792.

9. Bickell WH, Waal MJ, Pepe PE, et al. Immediate versus delayed fluid resuscitation for hypotensive patients with penetrating torso injuries. *New Engl J Med.* 1994; 331:1105-1109.

10. Shoemaker WC, Peitzman AB, Bellamy R, et al. Resuscitation from severe hemorrhage. *Crit Care Med.* 1996; 24:12S-23S.

11. Hambly PR, Dutton RP. Excess mortality associated with the use of a rapid infusion system at a level 1 trauma center. *Resuscitation.* 1996;31:127-133.

12. Dries DJ. Hypotensive resuscitation. *Shock.* 1996; 6:311-316.

13. Owens TM, Watson WC, Prough DS, Uchida T, Kramer GC. Limiting initial resuscitation of uncontrolled hemorrhage reduces internal bleeding and subsequent volume requirements. *J Trauma.* 1995; 39:200-207.

14. Capone AC, Safar P, Stezoski W, Tisherman S, Peitzman AB. Improved outcome with fluid restriction in treatment of uncontrolled hemorrhagic shock. *J Am Coll Surg.* 1995; 180:49-56.

15. Stern SA, Wang X, Mertz M, et al. Under-resuscitation of near-lethal

uncontrolled hemorrhage: Effects on mortality and end-organ function at 72 hours. *Shock.* 2001; 15:16-23.

16. Liu L-M, Ward JA, Dubick MA. Effect of crystalloid and colloid resuscitation on hemorrhage-induced vascular hyporesponsiveness to norepinephrine in the rat. *J Trauma.* 2003; 54. In press.

17. Michell MW, Rafie AD, Shah A, et al. Hypotensive and normotensive resuscitation resuscitation of hemorrhagic shock with Hextend or LR. *Crit Care Med.* 2003;12(suppl):A41.

18. Wu X, Stezoski J, Safar P, Tisherman SA. During prolonged (6 hours) uncontrolled hemorrhagic shock with hypotensive fluid resuscitation, MAP must be maintained above 60-70 mmHg in rats. *Crit Care Med.* 2003;12 (suppl):A40.

19. Stern SA, Dronen SC, Birrer P, Wang X. Effect of blood pressure on hemorrhage volume and survival in a near-fatal hemorrhage model incorporating a vascular injury. *Ann Emerg Med.* 1993; 22:155-163.

20. Kowalenko T, Stern S, Dronen S, Wang X. Improved outcome with hypotensive resuscitation of uncontrolled hemorrhagic shock in a swine model. *J Trauma.* 1992; 33:349-362.

21. Smith JG, Kramer GC, Perron P, et al. A comparison of several hypertonic solutions for resuscitation of bled sheep. *J Surg Res.*1985;39:517-528.

22. Wade CE, Hannon JP, Bossone CA, Hunt MM, et al. Resuscitation of conscious pigs following hemorrhage. Comparative efficacy of small volume resuscitation. *Circ Shock.* 1989; 29:193-204.

23. Hannon JP, Wade CE, Bossone CA, Hunt MM, Loveday JA. Oxygen delivery and demand in conscious pigs subjected to fixed-volume hemorrhage and resuscitated with 7.5% NaCl in 6% dextran. *Circ Shock.* 1989; 29:205-217.

24. Wade CE, Dubick MA, Vassar MJ, Perry CA, Holcroft JW. Plasma dextran concentrations in trauma patients administered HSD-70. *Clin Chem.* 1996; 42:779-780.

25. Mattox KL, Maningas PA, Moore EE, et al. Pre-hospital HSD infusion for post-traumatic hypotension. The USA multicenter study. *Ann Surg.* 1991; 213:482-491.

26. Vassar MJ, Perry CA, Gannaway WL, Holcroft JW. 75% sodium chloride/dextran for resuscitation of trauma patients undergoing helicopter transport. *Arch Surg.* 1991;126:1065-1072.

27. Dubick MA, Holcomb JB. A review of IO vascular access: Current status and military application. *Mil Med.* 2000; 165(7):552-558.

28. Calkins MD, Fitzgerald G, Bentley TB, Burris D. Intraosseous infusion devices: A comparison for potential use in special operations. *J Trauma.* 2000; 48:1068-1074.

29. Dubick MA, JW Pfeiffer, CB Clifford, Kramer GC. Comparison of IO and intravenous delivery of HSD in anesthetized, euvolemic pigs. *Ann Emerg Med.* 1992; 21:498-503.

30. Runyon DE, SP Bruttig, MA Dubick, Clifford CB, Kramer GC. Resuscitation from hypovolemia in swine with IO infusion of a saturated salt-dextran solution. *J Trauma.* 1994; 36:11-19.

31. Dubick MA, Kramer GC. Hypertonic saline dextran and IO vascular access for the treatment of hemorrhagic hypotension in the far-forward combat arena. *Annals Acad Med Singapore.* 1997; 26:64-69.

32. Hodge D III, Delgado-Paredes C, Fleisher G. Intraosseous infusion flow rates in hypovolemic "pediatric" dogs. *Ann Emerg Med.* 1987; 16:305-7.

33. Watson WC, Ryan DM, Dubick MA, Simmons DJ, Kramer GC. High pressure delivery of resuscitation fluid through bone marrow. *Academic Emerg Med.* 1995; 2:402.

34. Dubick MA, Sondeen JL, Szebeni J. Effects of hypotensive resuscitation with LR LR solution on complement activation after hemorrhage in swine. *FASEB J.* 2003; 17:A1237.

35. Roberts I, Evans P, Bunn F, Kwan I, Crowhurst. Is the normalization of blood pressure in bleeding trauma patients harmful? *Lancet.* 2001;357:385-387.

36. Henry S, Scalea TM. Resuscitation in the new millennium. *Surg Clin NA.*1999; 79:1259-1267.

37. Revell M, Porter K, Greaves I. Fluid resuscitation in pre-hospital trauma care: A consensus view. *Emerg Med J.* 2002;19:494-498.

38. Burris D, Rhee P, Kaufmann C, et al. Controlled resuscitation for uncontrolled hemorrhagic shock. *J Trauma.* 1999;46:216-223.

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Potential Resuscitation Strategies for the Treatment of Hemorrhagic Shock

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Exsanguination is the major cause of death on the battlefield. Of those who die on the battlefield, it is estimated that 20% could be salvaged before exsanguination if provided with immediate care. Upon arrival at the scene, a First Responder must immediately control bleeding. If the injury is on the body surface or extremity and compressible, direct pressure or a tourniquet is current standard treatment for attempting adequate hemostasis. Ideally, a hemostatic dressing would circumvent the tourniquet by staunching severe bleeding, and require no further attention by the medic. For suspected noncompressible bleeding, for which there is currently no adequate treatment, the ideal would be an intravenous resuscitation solution containing a substance that enhances clotting or clot stability only at the bleeding sites. Once bleeding is controlled, the next step is to resuscitate the patient.On the battlefield, if hemostasis is not assured, aggressive resuscitation may dislodge the clot and exacerbate bleeding; aggressive resuscitation also requires large volumes of fluid, presenting a logistical difficulty. An improved strategy would resuscitate only to the point at which survival was assured and would not cause further bleeding even during the predicted prolonged evacuations that may occur in an urban warfare environment. This article gives an overview of recent work using a severe hemorrhagic shock animal model with an arterial injury on (1) the point at which blood pressure dislodges the thrombus (the "pop-clot" pressure); (2) an injectable clot stabilizer ("fix-a-leak") that is a naturally occurring factor in the clotting cascade (human recombinant factor *VII); and (3) the maximum time up to 24 hours for hypotensive resuscitation below the "pop-the-clot" pressure ("how low for how long").*

Introduction

The concept that early, aggressive high-volume resuscitation is critical to the optimal treatment of hemorrhagic shock was widely accepted and practiced during the Vietnam War.[1] Subsequently, the practice of large volume crystalloid resuscitation became the standard of care for civilian trauma patients.[2] The foundation of this practice rests on the controlled hemorrhage studies conducted in the late 1960s and 1970s by Shires et al[3,4] The metabolic benefit of fluid resuscitation was definitively demonstrated in controlled hemorrhage animal models and then implemented in patients suffering uncontrolled hemorrhagic shock.[3]

However, numerous animal studies of uncontrolled hemorrhage have shown that there is increased blood loss following resuscitation induced by injury to blood vessels or organs.[5-18] Recent randomized clinical studies and a review of data collected during World Wars I and II similarly question the prudence of aggressive resuscitation in patients prior to hemorrhage control.[19-23] While the metabolic benefits of fluid resuscitation have long been recognized, these benefits must be balanced against the deleterious effects of rebleeding.[24-26] It is therefore essential to determine if there is a reproducible point at which rebleeding occurs. The optimal endpoint of resuscitation in patients with truncal injury without definitive hemorrhage control might then be just below this rebleeding point.

Our laboratory has developed an animal uncontrolled hemorrhage model that uses an injury in the aorta that closely approximates a severe arterial hemorrhage potentially encountered in the military setting. A round hole is made in the aorta with a skin biopsy punch that creates a wound profile of a ballistic or shrapnel fragment injury with actual loss of a piece of arterial wall. Using small punches, we can create an injury that spontaneously clots but, if rebleeding occurs, the additional hemorrhage will likely be fatal. Using large punches, we can create an injury that causes the animal to exsanguinate unless the hemostatic agent that we are testing is effective. In another article in this issue, Kheirabadi et al describes how this model has been modified to test dressings that would be effective with accessible compressible injuries. For this article, we will focus on reducing bleeding in noncompressible injuries.

Pop the Clot Pressure

The first study explored the possibility of a reproducible point of rebleeding, or a "pop-the-clot" pressure. In catheterized 40 kg anesthetized pigs in the supine position, through a midline incision in the abdomen, an initial aortotomy made with a 2 mm skin biopsy punch (Figure 1), simulated an injury to the aorta, and was allowed to clot. The unique feature of this experimental design is that all bleeding can be quantified because we have suction tubes in the abdomen. The aorta bleeds as it would in a real injury with the intestines over the aorta and injury. When the blood drains to the sides, the suction tubes take the blood into canisters that are on a balance and the weight of blood in the canister is recorded on a computer instantaneously. Figure 2 shows the blood pressure tracing in a representative experiment for the 2-hour experimental period with simultaneous measurement of resuscitation volume and hemorrhage volume.

To simulate varying times of arrival at the scene, we delayed aggressive resuscitation to 5, 15, or 30 minutes after the

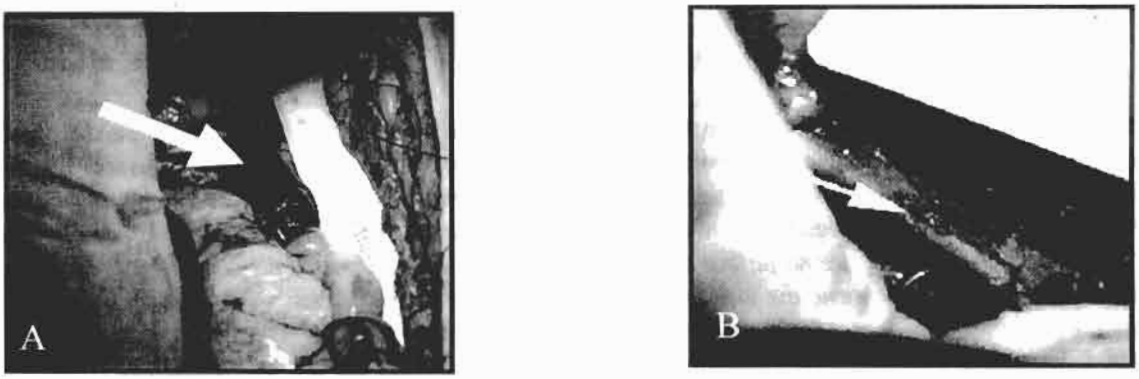

Fig 1. Abdominal aortotomy. A large central clot (white arrow) forms over the aorta to stop the initial bleeding. The majority of the blood has been suctioned into canisters and is not important in the initial hemostasis at the site of the clot (A). Postmortem, aortotomy size (arrow) is verified by exposing the site by clot removal (B). Interestingly, the gel clot (A) appeared to be the same even after rebleeding, so apparently, the clot was loosened with resuscitation, but not lost.

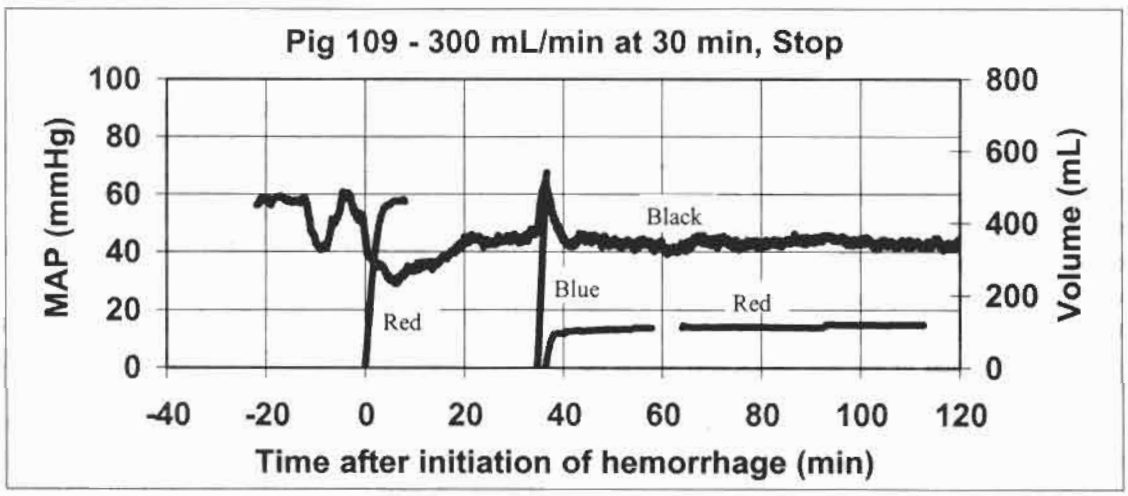

Fig 2. Blood pressure tracing over 2 hours measuring resuscitation volume and hemorrhage volume. Resuscitation was discontinued at rebleed. Baseline mean arterial pressure (MAP) was taken. Intestines were retracted and aorta exposed. This caused variable changes in the MAP reflected by a transient drop and recovery of the MAP prior to the aortotomy at time 0. The first red line denotes the aortotomy hemorrhage volume and spontaneous clotting at 5 minutes. Blood pressure spontaneously recovered to near stable value below baseline. Resuscitation at 30 minutes, resuscitation began with warmed lactated Ringer's (LR) (blue line). Rebleed MAP was determined by appearance of blood in canister after resuscitation (second red line). MAP; solution = LR. Red line = instantaneous hemorrhage volume; blue line = resuscitation volume; black line = MAP.

initial bleeding. We used two rates of resuscitation, 100 and 300 mL/min, with warmed LR solution (6 or 7 animals per group). The 300 mL/min rate is approximately that which can be delivered on the battlefield through a large-bore ($\leq$ 16 g) catheter using a manually inflated pressure bag. The resuscitation was continued until the clot was dislodged and the aortotomy rebled. The blood pressure at the point when blood appeared in the suction canister is designated as the rebleed pressure. We thought that, if the clot gained strength as it matured, we would find that the rebleed pressure would increase with time, or that the higher rate of resuscitation would cause rebleeding sooner. Instead, as can be seen in Table 1, there were no systematic significant changes, regardless of delay or rate of infusion. The rebleed blood pressure, averaged over all the groups, proved to be a reproducible MAP of 64 ± 2, a systolic pressure of 94 ± 3, and a diastolic pressure of 45 ± 2 mmHg.[27]

Rebleed MAP (mmHg)		
Delay (min)	**Rate (mL/min)**	
	100	300
5	70 ± 5	67 ± 5
15	57 ± 6	55 ± 5
30	63 ± 6	72 ± 4

Table 1. Mean Arterial Pressure at which Rebleeding Occurred in Response to Resuscitation with Warmed LR Solution at a Rate of Either 100 or 300 mL/min. The Resuscitation was Delayed 5, 15, or 30 Minutes from the End of the Initial Hemorrhage.

Continue or Stop Resuscitation after Rebleed Point

Although the primary question in the rebleed study was to determine whether there is a reproducible rebleed pressure, another question that could be asked is what happens if resuscitation is either continued to return blood pressure to baseline levels, or if the resuscitation is stopped to minimize rebleeding. The animals were re-randomized into "continue" or "stop" resuscitation groups once they rebled. At that point, resuscitation (either at 100 or 300 mL/min rate) was either continued until the MAP returned to pre-hemorrhage baseline levels or was discontinued. In the continue group, the resuscitation pump was turned off when the pressure was at baseline, or was turned back on until baseline pressure was obtained. In the stop group, no further resuscitation was given once rebleeding occurred and the animals were observed until death or for 2 hours. There was also a group of animals that received the aortotomy, but no resuscitation (negative control group).

As can be seen in Table 2, all three groups bled a similar volume from the initial aortotomy. In the continue resuscitation group, the hemorrhage continued and the rebleed hemorrhage volume was 4 times higher than the rebleed hemorrhage volume in animals in which blood pressure was not returned to baseline levels after rebleeding occurred. In addition, 5 times the volume of LR was used in the continue group compared with the stop group. Despite the large amount of additional fluid received by the continue group, survival time was not significantly affected (Table 2).

Figure 3 shows the pattern of bleeding in representative experiments in the continue group. In some animals, although not all of them, rebleeding continued as long as resuscitation was continued. Contrast this with Figure 2 in which rebleeding stopped as soon as the resuscitation was discontinued. However, the blood pressure remained well below baseline values. Despite the fact that the continue group received far more fluid than the stop group, survival was not improved (Table 2). Part of the reduced survival was a result of the design of the study in which aggressive resuscitation was used throughout and resulted in a very low hematocrit that, in and of itself, resulted in death. To prevent this, blood products are given as soon as possible in the emergency department. Only crystalloids and colloids are currently available on the battlefield, however, so we limited the choice of fluids to make the study relevant to the far-forward scenario.

The interesting result was that there was no worse or even slightly improved survival in the animals that received no resuscitation at all – and that had no additional loss of blood. This suggests that even a small amount of rebleeding was associated with decreased survival. However, the lack of any resuscitation also resulted in less than a 2-hour survival for the majority of the animals. These results agree with those from studies that investigated an arbitrary partial resuscitation with either a given volume or to an arbitrary blood pressure; these demonstrated improved survival with limited resuscitation.[7,12]

The finding of a reproducible rebleeding point suggests two strategies. One is to administer something that will stabilize the clot so that resuscitation to the baseline can be achieved while preventing further rebleeding. The other is to resuscitate to blood pressures below the rebleeding point. In the modern battlefield scenario, prolonged evacuation may occur due to wide dispersal of troops. Since others have demonstrated short-term (up to 4 hours) benefits of hypotensive resuscitation, the remaining question is whether hypotensive resuscitation with fluids currently used by the combat medic can sustain a subject for as long as 24 hours at a blood pressure less than the rebleeding point. If hypotensive resuscitation is not beneficial for as long as 24 hours, then it becomes even more important to: (1) stabilize the clot so that a higher pressure can be obtained

Variable	Treatment		
	No Resuscitation	Continue Resuscitation	Stop Resuscitation
	(n = 10)	(n = 16)	(n = 22)
Initial hemorrhage volume (mL/kg)	18 ± 2	16 ± 1	17 ± 1
Rebleed hemorrhage volume (mL/kg)	0 ± 0	29 ± 4**	7 ± 1*
Volume of LR administered (mL/kg)	0 ± 0	107 ± 15**	19 ± 3*
Survival time (minutes)	95 ± 13	85 ± 9	89 ± 8

*Table 2. Initial Hemorrhage Volume, Rebleed Hemorrhage Volume, Volume of LR Administered, and Survival Times in the No Resuscitation, Continue, and Stop Resuscitation Groups. ** Different from all Other Groups. *Different from No resuscitation*

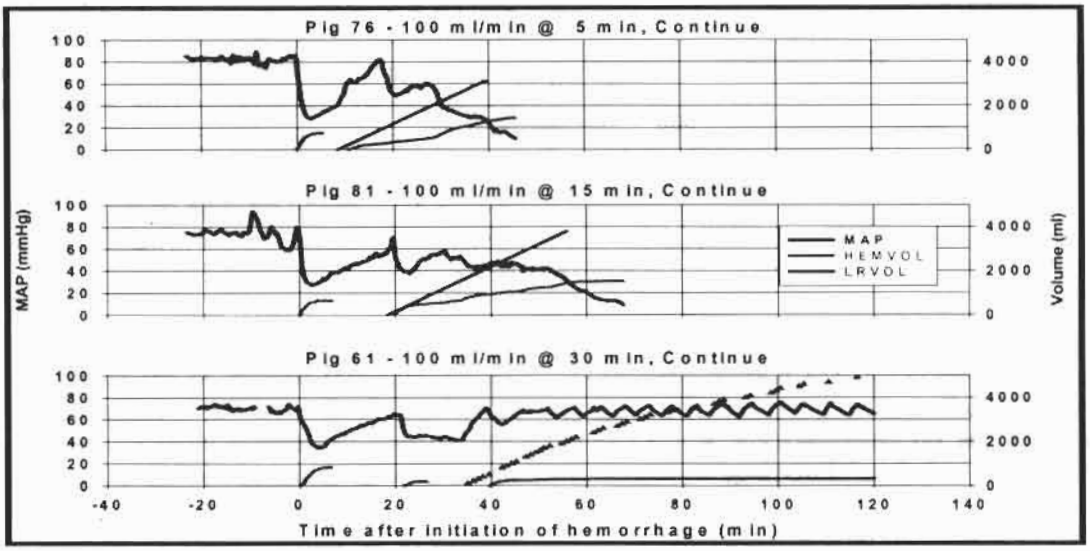

Fig 3. Three representative experiments of continued resuscitation after rebleed point. All three animals spontaneously recovered blood pressure following initial hemorrhage. After rebleeding, blood pressure fell and resuscitation continued for as long as blood pressure remained below baseline. Rebleeding continued as resuscitation continued if blood pressure did not be return to baseline (top 2 panels). This amount of resuscitation caused hematocrit to fall, and all resuscitation was stopped when the hematocrit reached 10%. Animal No. 61 spontaneously recovered to point of spontaneous rebleeding (bottom panel). Resuscitation was begun and the baseline blood pressure obtained. Resuscitation caused a third incidence of rebleeding, that did not continue despite continued resuscitation. The animal survived the entire 2 hours, hematocrit > 10%.

safely, or (2) develop better resuscitation solutions that will increase survival in a prolonged hypotensive state.

Stabilization of the Clot

Recombinant activated factor VII (rFVIIa) is an Food and Drug Administration (FDA) approved drug commonly utilized for the treatment of patients with hemophilia.[28,29] Attention has turned to its potential effectiveness in reducing bleeding in traumatic hemorrhage. Recent trauma studies have demonstrated its effectiveness in decreasing blood loss in models of hypothermic coagulopathic swine with grade V liver injuries.[30,31] A growing body of literature documents its successful use in surgical and trauma patients with the acquired coagulopathy of trauma.[32-34] The purpose of this study was therefore to determine whether administration of rFVIIa to a pig – with normal coagulation and an uncontrolled hemorrhage – would enhance clot stability and increase

rebleeding MAP in response to resuscitation.

In these experiments, the animal was prepared in a similar manner to the "pop-the-clot" study described above.[27] Five minutes before the aortotomy was made, an intravenous injection of either vehicle control, low dose (180 μg/kg) or high dose (720 μg/kg) rFVIIa was given. Five minutes after the injection was completed, the intestines were retracted and a 2.0 mm hole was made in the infrarenal aorta with a disposable skin biopsy punch. Ten minutes after the hole was made, resuscitation at 100 mL/min with LR solution at 37°C was begun. Rebleed pressure was determined by noting the blood pressure at the time blood appeared in the suction canister. If the MAP reached a plateau after 4 L of fluid were given without causing rebleeding, the LR pump was stopped and an infusion of epinephrine at 1.0 μg/kg/min, as needed, was given to raise MAP to as high as 200 mmHg. If no rebleeding occurred with this treatment, the animal was recorded as a nonrebleeder. The total volume of LR administered and the rebleed hemorrhage volume were recorded. Survival time up to 2 hours post aortotomy was recorded.

Pre-treatment with rFVIIa significantly increased the MAP at which rebleeding occurred during resuscitation of an uncontrolled hemorrhage from 53 ± 7 mmHg in the control group, to 71 ± 6 mmHg in the low dose group, and to 88 ± 17 mmHg in the high dose (P=0.05 between high dose and control). More resuscitation fluid volume (55 ± 12 mL/kg at the high dose) was given compared with the control (20 ± 9 mL/kg, $P \leq 0.005$) before rebleeding occurred. Resuscitation was given for a longer time (21 ± 5 min at the high dose) before rebleeding was induced compared with the control (8 ± 4 min, $P \leq 0.005$). There was a trend toward a reduced rebleed hemorrhage volume with rFVIIa, from 39 ± 9 ml/kg in control to 21 ± 7 mL/kg at the high dose, but it did not reach statistical significance (P=0.055). There was no reduction in the initial hemorrhage volume among the groups (22 ± 2, 20 ± 3, and 19 ± 2 mL/kg in the control, low, and high dose groups, respectively), despite high levels of circulating rFVIIa.

Although the reduction of rebleed hemorrhage volume with rFVIIa treatment did not reach statistical significance, there was a significant metabolic consequence from the increased blood loss in the control group leading to an elevated plasma lactate concentration compared with the 180 and 720 μg/kg groups and a trend toward a more negative base excess in the control group compared with the 180 and 720 μg/kg groups. The change in the arterial base excess was not due to changes in the ventilation since the animals were on a ventilator. Although not significant, the control group showed a trend toward a shorter survival time than the low and high dose FVII groups (73 ± 11, 87 ± 11, and 95 ± 11 min, respectively, P = 0.238).

A very interesting pattern emerged among the groups and this pattern is depicted in the representative experiments shown in Figure 4. In this model, the usual finding was that, once the thrombus has been disrupted, bleeding continued for as long as resuscitation was administered, as occurred in 70% of the animals in the control group (top panel, Figure 4 and in the pop-the-clot animals, Figure 3). Although there was rebleeding in the groups that received rFVIIa, this bleeding stopped, at least for a short time, in 100% and 88% of the low and high dose animals, respectively, despite continued resuscitation (middle and bottom panels, Figure 4).

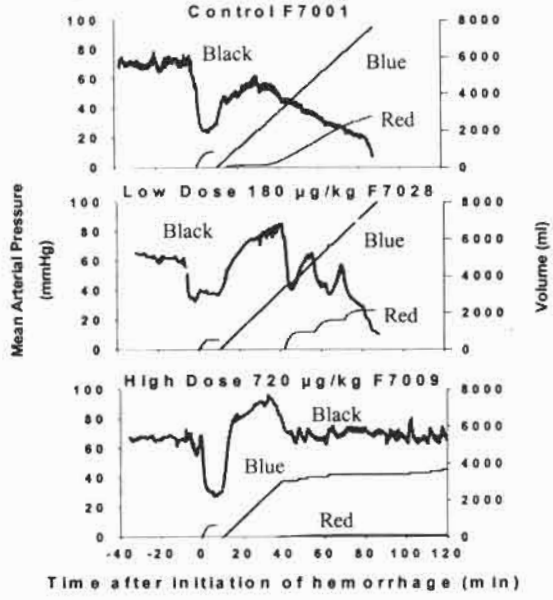

Fig 4. Control, low and high dose rFVIIa experiments: intermittent rebleeding with rFVIIa despite continuing resuscitation. MAP; LR solution. Red line = instantaneous hemorrhage volume; blue line = resuscitation volume; black line = MAP

Interestingly, rFVIIa pretreatment in the current study provided no hemostatic benefit in reducing the initial hemorrhage volume. This may indicate that the presence of rFVIIa has no measurable effect when the blood flows are high as they are in the pigs at baseline. Similar results were obtained by Schreiber et al, who treated pigs with rFVIIa 30 seconds after the induction of the liver injury and began resuscitation 15 minutes after the injury.[31] At high blood flows in the normotensive subject, the shear forces may therefore prevent platelets and other factors from concentrating at the site of injury. During the hypotension following hemorrhage, platelets may be able to collect at the injured site and a rapid, full thrombin burst may help to form a more stable clot with a firm fibrin structure that can better resist dislodging when normal rates of flow are reestablished following resuscitation.

Promising preliminary results from a group in England suggest that rFVIIa has a significant effect on survival time and

hemorrhage volume in their model of combined controlled-uncontrolled hemorrhage. The rFVIIa was given just before an aortotomy was made and resuscitation begun, during a short period between the end of the controlled hemorrhage and the start of the uncontrolled phase. The animals were then given either full resuscitation to Advanced Trauma Life Support standards or hypotensive resuscitation to a systolic pressure of 80 mmHg. The different resuscitation methods (complete vs hypotensive) showed a tendency towards a beneficial effect for hypotensive resuscitation that was most pronounced when the rFVIIa was combined with hypotensive resuscitation (conversation with Wayne Sapsford, Defense Science and Technical Laboratories, Porton Down, UK).

Hypotensive Resuscitation

As mentioned previously, hypotensive resuscitation to arbitrary endpoints has been shown to reduce bleeding in uncontrolled hemorrhage models, at least in the short-term. We are currently conducting experiments to determine if resuscitation to a systolic blood pressure of 80 mmHg can be sustained for 24 hours. We chose a systolic pressure of 80 mmHg because it is below the "pop-the-clot" systolic pressure of 94 mmHg; additionally, it is the pressure at which a radial pulse can be detected and is therefore an appropriate target achievable on the battlefield. We are comparing various fluids that are either FDA-approved , or are undergoing application to the FDA for approval, for their efficacy under conditions of this hypotensive resuscitation. The fluids are LR solution, 6% hetastarch in a LR base (Hextend ™), and a hemoglobin-based oxygen carrier (Polyheme ™). The questions we are asking are (1) whether there is rebleeding during the hypotensive period; (2) whether the animals can tolerate prolonged hypotension; and (3) and which fluid provides the best metabolic support with the least volume. It is possible that the prolonged hypotension might cause some tissues to be relatively ischemic, so we are also taking samples to assess tissue function, oxidative, and nitrative states, and coagulation status. To see if there are changes in the synthesis of heretofore unknown metabolites, we are also performing genetic microarray analysis of the white blood cell response over the 24-hour experimental course as is described in other papers found in this issue (Dubick and Bowman). At the end of the 24 hours, we repair the aortotomy and then let the animal recover for an additional 2 days to ensure that multiple organ dysfunction does not develop. Based on acute studies and short-term clinical trials, the recommendations for hypotensive resuscitation has been promulgated for the special operations medics[19,20,35,36]

Future Directions

The large animal, severe hemorrhagic shock models that we have been studying also allow us to investigate endpoints of resuscitation that are more sensitive than blood pressure. For example, in a series of nonresuscitated animals that bled different volumes in response to injury, three survival patterns emerged: those who survived for less than 1 hour, those who survived between 1 and 2 hours, and those who lived for the entire 2 hours (unpublished observations). Noninvasive and metabolic data from these experiments may yield new endpoints of resuscitation that may be early warning signs of impending circulatory collapse.

The normal response to severe hemorrhage when bleeding stops is for the blood pressure to spontaneously increase as the animal's intrinsic compensatory mechanisms start to operate. As can be seen in Figure 5, those animals that did not start to increase their blood pressure within 10 minutes did not survive a full hour. The other two groups did compensate with similar increases in blood pressure, yet one group succumbed earlier than the other. By looking at other endpoints, we found that large changes in lactate and arterial base excess as early as 15 minutes after injury distinguish between those who die early and those who survive.

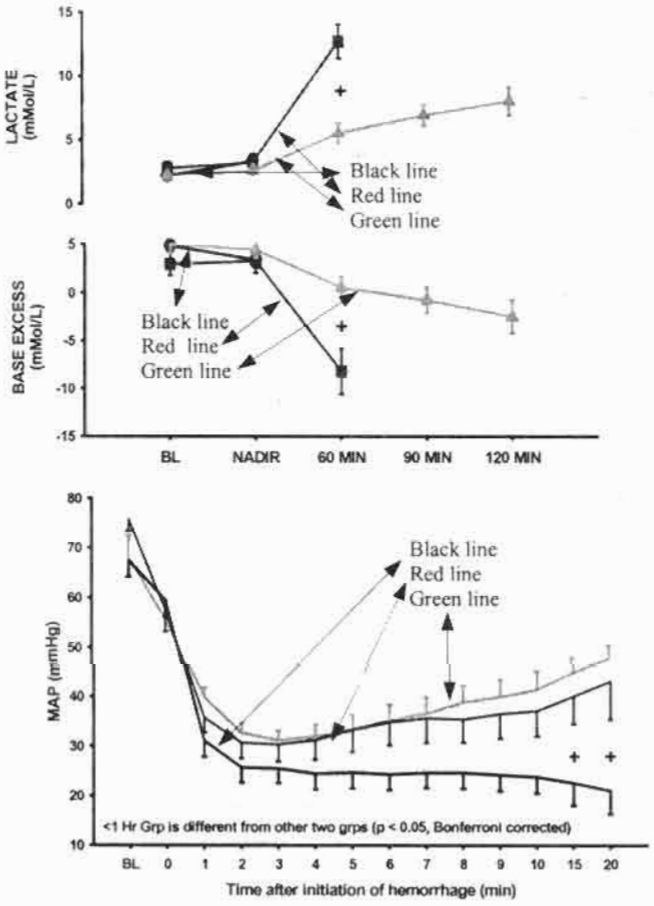

Fig 5. MAP in three groups of non-resuscitated pigs. Death ≤ 1 hour = black line; Death at 1-2 hours = red line; Survival > 2 hours = green line. Early changes in arterial lactate and base excess may differentiate between survivors and no-survivors although blood pressures are similar.

Because of studies and results like these, we feel that strategies to develop rugged instruments and realistic decision assist algorithms can be developed to better help the medic perform triage in the far-forward environment.

References

1. Medicine Io, ed. *Fluid Resuscitation: State of the science for treating combat casualties and civilian injuries*. Washington, DC: National Academy Press; 1999.

2. Pepe PE, Mosesso VN, Jr, Falk JL. Prehospital fluid resuscitation of the patient with major trauma. *Prehosp Emerg Care*. 2002;6:81-91.

3. Shires GT, Carrico CJ, Canizaro PC. *Shock*. vol XIII. Philadelphia: W. B. Saunders Company; 1973:p 119 -122.

4. Stern SA. Low-volume fluid resuscitation for presumed hemorrhagic shock: helpful or harmful? *Curr Opin Crit Care*. 2001;7:422-430.

5. Bickell WH, Bruttig SP, Millnamow GA, O'Benar J, Wade CE. The detrimental effects of intravenous crystalloid after aortotomy in swine. *Surgery*. 1991;110:529-36.

6. Bickell WH, Bruttig SP, Millnamow GA, O'Benar J, Wade CE. Use of hypertonic saline/dextran versus LR solution as a resuscitation fluid after uncontrolled aortic hemorrhage in anesthetized swine. *Ann Emerg Med*. 1992;21:1077-85.

7. Burris D, Rhee P, Kaufmann C, et al. Controlled resuscitation for uncontrolled hemorrhagic shock. *J Trauma*. 1999;46:216-23.

8. Elgjo GI, Knardahl S. Low-dose hypertonic saline (NaCl 8.0%) treatment of uncontrolled abdominal hemorrhage: effects on arterial versus venous injury. *Shock*. 1996;5:52-8.

9. Gross D, Landau EH, Klin B, Krausz MM. Quantitative measurement of bleeding following hypertonic saline therapy in "uncontrolled" hemorrhagic shock. *J Trauma*. 1989;29:79-83.

10. Gross D, Landau EH, Klin B, Krausz MM. Treatment of uncontrolled hemorrhagic shock with hypertonic saline solution. *Surg Gynecol Obstet*. 1990;170:106-12.

11. Holmes JF, Sakles JC, Lewis G, Wisner DH. Effects of delaying fluid resuscitation on an injury to the systemic arterial vasculature. *Acad Emerg Med*. 2002;9:267-74.

12. Kowalenko T, Stern S, Dronen S, Wang X. Improved outcome with hypotensive resuscitation of uncontrolled hemorrhagic shock in a swine model. *J Trauma*. 1992;33:349-53. discussion 361-2.

13. Krausz MM, Landau EH, Klin B, Gross D. Hypertonic saline treatment of uncontrolled hemorrhagic shock at different periods from bleeding. *Arch Surgery*. 1992;127:93-6.

14. Leppaniemi A, Soltero R, Burris D, et al. Fluid resuscitation in a model of uncontrolled hemorrhage: too much too early, or too little too late? *J Surg Res*. 1996;63:413-8.

15. Marshall HP, Jr, Capone A, Courcoulas AP, et al. Effects of hemodilution on long-term survival in an uncontrolled hemorrhagic shock model in rats. *J Trauma*. 1997;43:673-9.

16. Matsuoka T, Hildreth J, Wisner DH. Liver injury as a model of uncontrolled hemorrhagic shock: resuscitation with different hypertonic regimens. *J Trauma*. 1995;39:674-80.

17. Riddez L, Drobin D, Sjostrand F, Svensen C, Hahn RG. Lower dose of hypertonic saline dextran reduces the risk of lethal rebleeding in uncontrolled hemorrhage. *Shock*. 2002;17:377-82.

18. Sindlinger JF, Soucy DM, Greene SP, Barber AE, Illner H, Shires GT. The effects of isotonic saline volume resuscitation in uncontrolled hemorrhage. *Surg Gynecol Obstet*. 1993;177:545-50.

19. Martin RR, Bickell WH, Pepe PE, Burch JM, Mattox KL. Prospective evaluation of preoperative fluid resuscitation in hypotensive patients with penetrating truncal injury: a preliminary report. *J Trauma*. 1992;33:354-61. discussion 361-2.

20. Bickell WH, Wall MJ, Jr, Pepe PE, et al. Immediate versus delayed fluid resuscitation for hypotensive patients with penetrating torso injuries. *New Engl J Med*. 1994;331:1105-9.

21. Dutton RP, Mackenzie CF, Scalea TM. Hypotensive Resuscitation during Active Hemorrhage: Impact on In-Hospital Mortality. *J Trauma*. 2002;52:1141-6.

22. Cannon WB. *Traumatic Shock*. New York: Appleton and Company; 1923.

23. Beecher HK. *Resuscitation and Anesthesia for Wounded Men; The Management of Traumatic Shock*. Springfield, IL: Charles C. Thomas; 1949:124-126.

24. Henry S, Scalea TM. Resuscitation in the new millennium. *Surg Clin North America*. 1999;79:1259 - 1267.

25. Revell M, Porter K, Greaves I. Fluid resuscitation in pre-hospital trauma care: a consensus view. *Emerg Med J* 2002;19:494 - 498.

26. Santibanez-Gallerani AS, Barber AE, Williams SJ, Zhao BSY, Shires GT. Improved survival with early fluid resuscitation following hemorrhagic shock. *World J Surgery*. 2001;25:592-7.

27. Sondeen JL, Coppes VG, Holcomb JB. Blood pressure at which rebleeding occurs after resuscitation in swine with aortic injury. *J Trauma*. 2003. In press.

28. Hedner U. Recombinant activated factor VII as a universal haemostatic agent. *Blood Coag Fibrinol*. 1998;9:S147-52.

29. Hedner U, Ingerslev J. Clinical use of rFVIIa. *Transfus Sci*. 1998;19:163-76.

30. Martinowitz U, Holcomb JB, Pusateri AE, et al. Intravenous rFVIIa administered for hemorrhage control in hypothermic coagulopathic swine with grade V liver injuries. *J Trauma*. 2001;50:721-9.

31. Schreiber M, Holcomb J, Hedner U, et al. The effect of rVIIa on non-coagulopathic pigs with grade V liver injuries. *J Surg Res*. 2002. In press.

32. Holcomb J, Neville L, Fischer C, Hoots K. Use of rFVIIa for intraperitoneal coagulopathic bleeding in a septic patient. *Curr Surg*. 2002. In press.

33. Martinowitz U, Kenet G, Segal E, et al. Recombinant activated factor VII for adjunctive hemorrhage control in trauma. *J Trauma*. 2001;51:431-8. discussion 438-9.

34. O'Neill PA, Bluth M, Gloster ES, et al. Successful use of recombinant activated factor VII for trauma-associated hemorrhage in a patient without preexisting coagulopathy. *J Trauma*. 2002;52:400-5.

35. Pepe PE, Wyatt CH, Bickell WH, Bailey ML, Mattox KL. The relationship between total prehospital time and outcome in hypotensive victims of penetrating injuries. *Ann of Emerg Med.* 1987;16:293-7.

36. Butler FK, Jr., Hagmann JH, Richards DT. Tactical management of urban warfare casualties in special operations. *Mil Med.* 2000;165:1-4.

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The Application of Genomics to the Battlefield: Microarrays and Gene Expression Analysis

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Baiteng Zhao, PhD††
Jill Sondeen, PhD†††

Introduction

Although hemorrhage due to combat injuries is the principal cause of death of soldiers on the battlefield, we know little about how the body responds to loss of blood and which organs are most affected.[1] While we know that a drop in blood pressure below about 30 mmHg or loss of more than 50% of the blood volume is tantamount to death, most cells in the body, with the exception of brain cells, are alive for several hours after injury despite receiving little oxygen or nutrients. This fact is used to advantage by organ transplant surgeons who can transplant a variety of organs up to several days after the death of the donor if proper storage protocols were followed. All mammals studied thus far exhibit similar responses to hemorrhage as they attempt to conserve resources and repair the damage caused by the loss of blood. If the injury is not too severe and is uncomplicated by infection, recovery is the usual outcome. Above a certain level of injury, however, most injured will die due to an exaggerated inflammatory response or complications of hemorrhagic shock. Saving the lives of this last class of injured requires a better understanding of these processes, especially the processes involving production of damaging factors by cells in response to the hemorrhagic insult. Currently, the most efficient and comprehensive method for understanding the responses of an organism to injury are with microarrays.

Microarrays and How They Work

Genomics is a new field that has grown out of the ability to sequence the deoxyribonucleic acid (DNA) that encodes each organism – the genome – and its goal is to define the genes in an organism and determine their function. Although each cell in the body contains a complete set of instructions for specifying all the functions of the body, only a limited amount of this genetic material is active, or expressed, and the portions of the genome that are active are specific for each cell type. The repertoire of the thousands of genes that are expressed in each cell type is termed the transcriptome. Until recently, traditional molecular techniques allowed analysis of only one gene at a time. Such limited throughput precluded an accurate picture of the molecular players that orchestrate the regulation of health or the dysregulation occurring in disease or following injury such as blood loss. Microarray technology, which allows analysis of changes in expression of thousands of genes, promises to help clarify the molecular and genetic basis of health and disease and speed drug discovery.

Microarrays consist of thousands of fragments of genes that are packed into several square centimeters by computer-controlled robots. Also known as DNA or gene chips, microarrays represent the first widely used biological application built upon the information provided by genome sequencing projects. The genetic code can be thought of as code based upon four letters. In practice, sequences of 50-70 nucleotides are spotted onto most in-house produced microarrays (Figure 1) and each spot, by reference to the genomic database, uniquely describes a gene. The technique for making gene chips was first introduced by the biotechnology company Affymetrix (Santa Clara, CA) which synthesizes short oligonucleotides onto a glass substrate by a photolithographic process.[2,3] Most laboratories produce their own chips by spotting preformed copy DNA (cDNA), or oligonucleotides, by a technique developed by Patrick Brown's laboratory at Stanford University.[4-6] During the course of a study, samples of blood or tissues are collected at specified times. Then the ribonucleic acids (RNA) from the sample is isolated and a copy

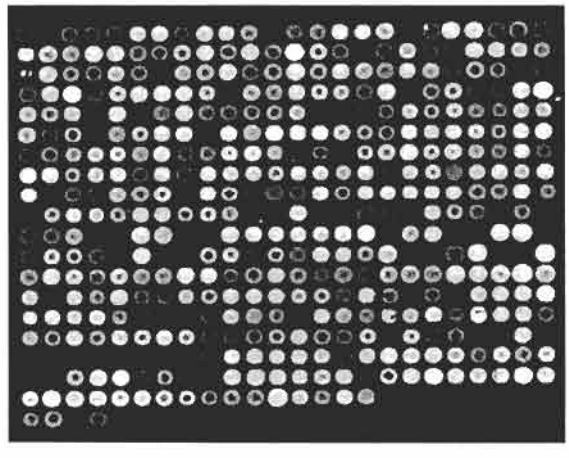

Fig 1. An example of a subgrid from a microarray used for quantifying gene expression from rat tissues. Yellow = no change in expression; green = downward expression; red = upward regulation of gene expression.

made with an enzyme that can generate cDNA. This cDNA is allowed to bind with the complement attached to the chip. After removal of unbound material, any bound material is scanned and quantified by a fluorescent scanner to detect sites of molecular hybridization to detect genes expressed by the cells under investigation at the time the messenger RNA (mRNA) was isolated. This application of microarrays is termed gene expression analysis. If one wants to know the influence of a drug or disease on the activity of many genes, gene expression analysis is one of the least expensive and most robust technique currently available. By combining this technology with computers that can track and record their activity, thousands of genes can be followed simultaneously.

One example of the use of microarray technology applied to the problem of hemorrhagic shock and resuscitation at the U.S. Army Institute of Surgical Research is the study of the genetic responses to 40% hemorrhage in rat and mouse models. While we plan to examine several organs and tissues in these animals, we are focusing first on lung as it is the predominant organ to fail in humans after severe trauma.[7] In animal models, hemorrhage even in distant organs results in lung injury.[8-11] Figure 2 illustrates the steps in performing gene expression analysis with microarrays in rodents.

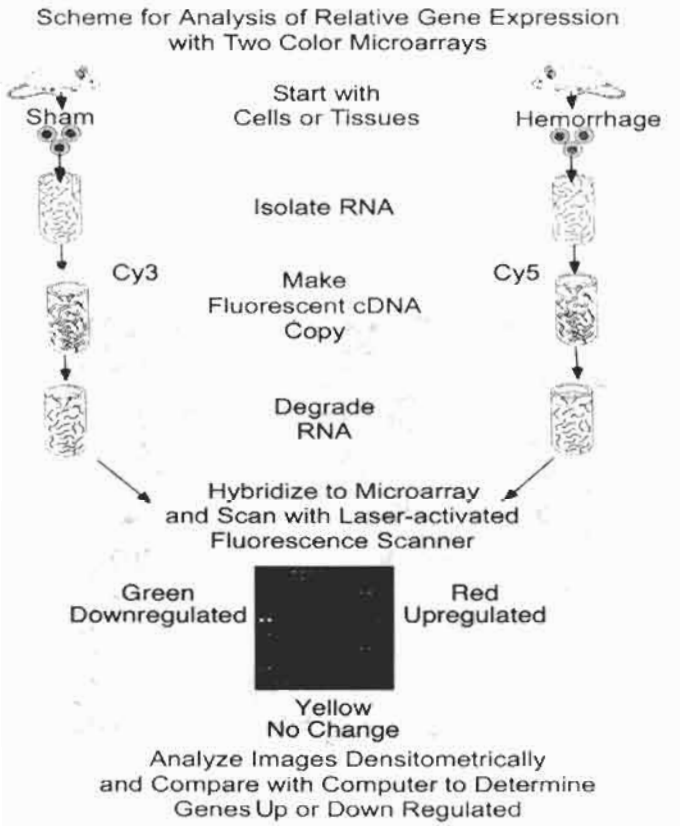

Fig 2. The steps in performing gene expression analysis with microarrays.

Figure 1 shows a small array of 480 nucleic acid fragments produced at the U.S. Army Institute of Surgical Research. Each spot is about 120 microns (0.12 mm) in diameter, and was deposited by a stainless pin in a special computer controlled robot. The exact order of each spot is tracked by appropriate software on the computer. About 80,000 spots can be produced on a standard microscope slide. Although there is still controversy about the exact number of genes (estimates are currently about 40,000) in mammalian genomes, representative fragments of the entire genome can, in theory, be placed on microarrays and all genes analyzed simultaneously. The RNAs, the immediate products of genes, are the effectors of the transcriptome. The RNAs are isolated and copies made (cDNA) that incorporate a fluorescent dye. When hybridized to the array, each cDNA finds its appropriate complementary sequence on the array, roughly in proportion to its concentration in the cell. By quantifying the fluorescence in a laser-activated scanner, the quantity of RNA present in the original mixture can be determined. In practice, an appropriate control from organs or cells that have not been perturbed is labeled with one color fluorescent dye while the experimental sample is labeled with a different colored dye.

The tools for handling the large data sets generated by microarray technology are in development and constantly improving. The principal tool currently in use is known as cluster analysis; it organizes data on the basis of some pattern. Figure 3 illustrates the results of alterations in gene expression in

Fig 3. Dendrogram of the results of cluster analysis. Thirty-seven genes were upregulated at least 6-fold at 2 time points in nonresuscitated rats during 72 hours following hemorrhage.

the lung of rats after a 40% reduction in blood volume as a function of time. The cluster analysis program associated genes whose expression was altered together as a function of time after hemorrhage.

Many of the genes altered in rat lung are termed expressed sequence tags. These are legitimate genes that are expressed in a variety of cell types, the specific functions of which are as yet unknown. For example, in rat lung, about 200 genes out of 5,800 spotted on the microarray are altered two-fold or more following hemorrhage. The goal of using gene expression analysis in developing resuscitation fluids is to use this genetic information in determining if a particular resuscitation fluid is either reducing the shock response or accelerating the early return to the pre shock state.

In conclusion, microarray technology is proving to be an extremely useful tool in discovering the underlying alterations that occur in cells and tissues as a consequence of hemorrhage and has direct application to other forms of injury such as burn, blunt trauma, sepsis, and head injury, all important forms of injury that are found on the battlefield. The application of this technology to understanding the responses of mammals to injury at the molecular level will lead to development of new drugs and treatments to counteract the deleterious affects of injury in humans.

References

1. Bellamy RF. The causes of death in conventional land warfare: implications for combat casualty care research. *Mil Med.* 1984;149(2): 55-62.

2. Fodor SP, Rava RP, Huang XC, Pease AC, Holmes CP, Adams CL. Multiplexed biochemical assays with biological chips. *Nature.* 1993;364 (6437): 555-6.

3. Fodor SP, Read JL, Pirrung MC, Stryer L, Lu AT, Solas D. Light-directed, spatially addressable parallel chemical synthesis. *Science.* 1991;251(4995): 767-73.

4. Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science.* 1995; 270(5235): 467-70.

5. Schena M, Shalon D, Heller R, Chai A, Brown PO, Davis RW. Parallel human genome analysis: Microarray-based expression monitoring of 1000 genes. *Proc Natl Acad Sci USA.* 1996; 93(20):10614-10619.

6. DeRisi J, Penland L, Brown PO, et al. Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat Genet.* 1996;14(4): 457-60.

7. Faist E, Baue AE, Dittmer H, Heberer G. Multiple organ failure in polytrauma patients. *J Trauma.* 1983; 23(9): 775-87.

8. Abraham E, Jesmok G, Tuder R, Allbee J, Chang YH. Contribution of tumor necrosis factor-alpha to pulmonary cytokine expression and lung injury after hemorrhage and resuscitation. *Critical Care Medicine.* 1995;23(8): 1319-26.

9. Shenkar R, Cohen AJ, Vestweber D, Miller YE, Tuder R, Abraham E. Hemorrhage and resuscitation alter the expression of ICAM-1 and P-selectin in mice. *J Inflamm.* 1995; 45(4): 248-59.

10. Shenkar R, Schwartz MD, Terada LS, Repine JE, Mccord J, Abraham E. Hemorrhage activates NF-kappa B in murine lung mononuclear cells in vivo. *Amer J Physiol-Lung Cell M Ph* .1996; 14(5): L729-L735.

11. Shenkar R, Abraham E. Hemorrhage induces rapid in vivo activation of CREB and NF-kappaB in murine intraparenchymal lung mononuclear cells. *Am J Respir Cell Mol Biol.* 1997;16(2): 145-52.

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Advanced Diagnostics for the Combat Medic

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Introduction

The U.S. Army Institute of Surgical Research (USAISR) has the lead for directing the Research Task Area for Advanced Diagnostics and Triage for the MRMC research program in Combat Casualty Care. The objective of this Task Area is to develop and demonstrate a semi-automated trauma triage capability that provides critical casualty information remotely to the battlefield medic. When this goal is met, the medic will possess a greater decision-making capability for prioritizing casualty care based on continuous information about live/dead status and severity and progression of the injury and which injuries require lifesaving interventions (LSI). Since hemorrhagic shock remains a leading cause of death on the battlefield, the research activities in the task area for advanced diagnostics and triage are designed to focus on the identification and care of wounded soldiers with severe hemorrhage. This Research Task Area is founded on the fundamental premise that meeting this goal will save lives on the battlefield. The purpose of this article is to describe the Task Area plan for conducting research that will lead to advanced diagnosis and triage capabilities for the combat medic by developing an algorithm for clinical assessment of wounded soldiers.

Background

Acute hemorrhage and subsequent circulatory collapse (shock) account for about 50% of the deaths on the battlefield and the forward operating table, a statistic that has remained relatively unchanged since World War I.[1] In addition, hemorrhage is the primary cause of death in about 30% of the injured soldiers who die from wounds. Likewise, uncontrolled hemorrhage accounts for up to 82% of the early operative deaths from trauma in the civilian arena. However, the mortality rate in combat casualties drops to 2% to 4% if the trauma patient is stabilized through surgery.[1,2] It is therefore clear that the ability to significantly reduce the mortality and morbidity associated with hemorrhagic shock on the battlefield will depend heavily on improving the capability of first level responders (medics) to apply early LSI.

Hemorrhagic shock is typically identified by the degree of hypotension and nonspecific signs and subjective symptoms such as cold clammy skin, pallor, weak thready pulse, unstable vital signs, and diminished mentation that develop as a result of blood loss.[3] There are several physiological measures that predict circulatory shock and subsequent poor outcome. Of these, the battlefield medic is currently limited to the assessment of mental status, pulse character and pulse rate measurements for diagnosis of wounded soldiers. In special operation forces, it is rare that standard blood pressure (BP) and pulse oximetry may be available. Although significant reductions in BP and oxygen carrying capacity of the blood (PaO_2), and elevations in heart rate (HR) can be measured in the civilian arena and are routinely used to assess progression toward circulatory collapse, compensatory mechanisms that buffer against changes in BP and PaO_2 make these measurements poor predictors for early assessment of shock.[3,4] This notion was supported by preliminary data from our laboratory demonstrating that arterial oxygen (O_2) saturation and BP changed very little (Figure 1, panels A and B) during a significant (as much as 2 liters) gradual reduction in central blood volume in humans that caused dramatic reductions in stroke volume and cardiac output (Figure 1, panels D and E). In addition, elevated HR (Figure 1, panel C) in a wounded soldier may be impossible to accurately interpret since "fight-or-flight" responses are a natural consequence of battle. Therefore, a definition based on the absence or presence of hypotension as measured by changes in mental status, pulse character, and/or HR can be misleading since it does not represent the underlying problem of, or the solution to, hemorrhagic shock.

Monitoring for the onset of circulatory shock in a civilian trauma patient has also focused on the clinical "gold standard" assessments of BP, arterial O_2 saturation, or simple pulse palpation (rate and character). Unfortunately, these measurements in a wounded soldier on the austere battlefield environment probably will be even more imprecise, subjective, and inconsistent. More important, the appearance of hypotension and other signs and symptoms of shock do not mark the beginning of circulatory compromise, but rather represent the beginning of decompensation (a point in time when it may be too late to introduce effective LSI). This notion was reaffirmed from a preliminary study performed from the USAISR animal database. With the use of specific data mining and multivariate regression analysis, it was demonstrated that the mean arterial pressure was a predictor of cardiovascular collapse but that the predictive power gave too little response time to be useful to a combat medic performing triage and resuscitation (J Ward, unpublished data).

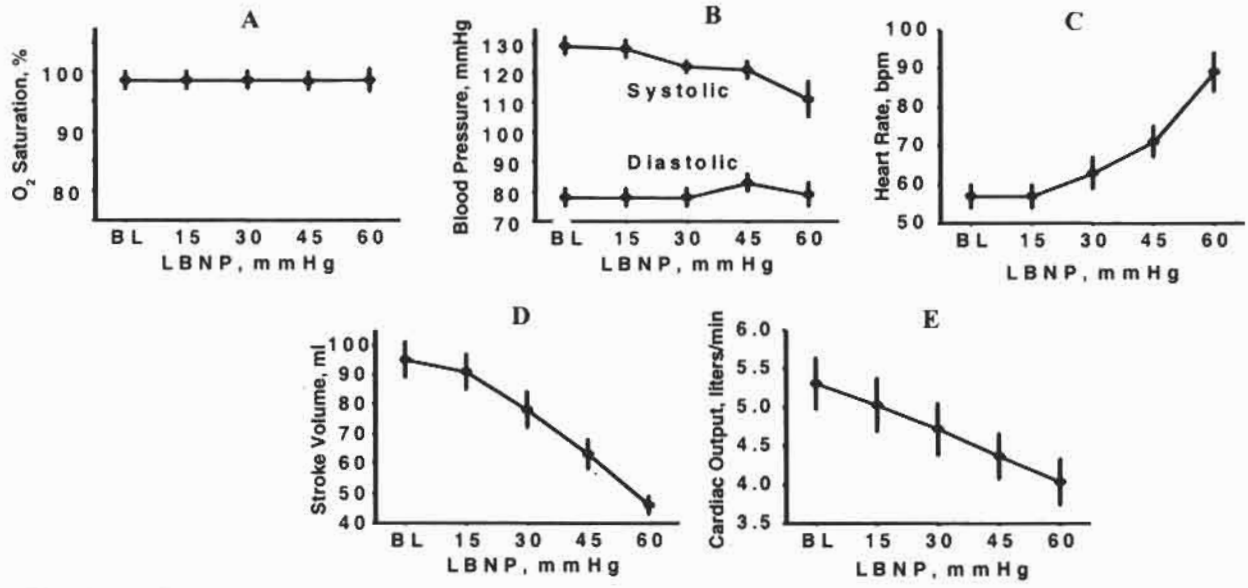

Fig 1. Hemodynamic and arterial O_2 saturation responses to graded reductions in central blood volume. The LBNP was used to transiently pool blood in the legs, thereby creating central hypovolemia in intathumans. Values are mean ± 1 standard error.

Since the appearance of hypotension and reduced PaO_2 reflect late events in the process of hemorrhagic shock, it is critical to identify physiological signals that will be altered during the earliest time period of blood volume loss. A common denominator in development of shock is the inadequate oxygen delivery (DO_2) to the tissue that is associated with reductions in blood flow (cardiac output) or metabolic alterations (reduced pH or base excess). Increased cardiac output and DO_2 correlate well with survival while failure to stabilize cardiac output and DO_2 is highly correlated with death.[4-8] Therefore, an algorithm that includes some indicator of DO_2 (for example, stroke volume, cardiac output) may represent a better tool for the early prediction of circulatory shock then measurements currently used for this purpose.

Describing Triage Challenges to the Medic

In the Objective Force Warrior battlefield environment, soldiers will be widely dispersed, being separated by time and distance from medic and/or buddy aid. Independence of objective force operations places a requirement on far-forward treatment, stabilization, and maintenance of wounded soldiers with technology that provides moment-to-moment real time monitoring of sensitive predictors for the onset of hemorrhagic shock and requirements for LSI. Large gains can be potentially achieved far-forward by simplifying and improving initial assessment of injury, appropriate intervention, and priorities for early evacuation.

Optimal management designed to prevent the onset of circulatory shock requires a recognition and integration of multiple complex physiological responses with varying time courses. The resulting challenge is that shock is easily diagnosed in late stages when therapy is ineffective while early diagnosis is difficult in the absence of measurements that represent physiological responses associated with the underlying mechanisms of shock. The solution to this dilemma is to identify the physiologic signal(s) that provides the best early indicators of blood volume loss and impending circulatory collapse. Such requirements for complicated information and decision-making can overwhelm a physician well trained in critical care medicine much less a first level responder (medic). Human capabilities for making the most appropriate and timely decisions for application of an effective LSI can be augmented by new technologies that provide automated data mining, trending, and decision support software. Previous efforts in this direction have centered upon developing hardware for casualty assessment. However, before developing hardware, an effective database of multiple physiologic signals associated with BP regulation must be constructed and evaluated to identify the best early predictors of impending cardiovascular collapse. Development of the optimal hardware (medical monitoring devices) will depend on validating an algorithm that identifies primary predictive physiological signals. This algorithm should provide the medic with essential, continuous information about the severity and clinical progression of the casualty and remote triage decision-making for prioritization of care and evacuation. Therefore, the result of the research in this Task Area should significantly enhance the decision-making capability of the medic and subsequently improve casualty outcome on the battlefield.

The physiology of the injured soldier suffering from severe hemorrhage is very dynamic, yet pre-evacuation care and monitoring have traditionally been based on isolated measurements even under the best circumstances. The absence of frequent physiological measurements obtained from the wounded soldier forces battlefield medics to make rapid decisions about priority of care and application of interventions based upon isolated "snapshot" data points (for example, BP, pulse character, respiratory rate, mental status) without the benefit of observing trends and the dynamic nature of the evolving trauma physiology. Thus, the current process of combat casualty care can be greatly improved by providing appropriate, continuous physiological observations. In support of this concept, data from civilian trauma literature shows that temporal patterns of physiological responses during hemorrhage are more informative than single measurements because they provide a history of physiologic events that lead to shock.[3] It is, therefore, clear that identification of the best early predictors of hemorrhagic shock can only be accomplished by simultaneous and continuous measurement of various physiological signals (responses) associated with BP regulation that have been proven to be accurate predictors of cardiovascular collapse.

Research Activities in Advance Diagnosis for the Combat Medic

Realizing the limits of the current triage capabilities (absence of critical continuous measurements such as BP, cardiac output, and DO_2), an algorithm that performs or facilitates remote triage on the battlefield is one of the primary objectives of current Combat Casualty Care research. Such a reliably predictive algorithm does not currently exist but is critically needed. The focus of the research conducted in the Task Area on Advance Diagnosis for the Combat Medic will therefore be placed on the development of an extensive database of multiple physiological responses to models of central hypovolemia in both humans and animals. By defining the outcome variable as time required to reach cardiovascular collapse, the resulting database should provide the foundation for development of an algorithm capable of predicting the need for a LSI. In order to develop an accurate triage algorithm that predicts the onset of cardiovascular collapse, it will be necessary to collect numerous physiologic signals simultaneously in models of central hypovolemia (hemorrhage). The USAISR has three general models of cardiovascular collapse (hemorrhagic shock) in animals and humans from which extensive databases are being developed.

Animal Hemorrhage Models. A number of large and small animal models of controlled and uncontrolled hemorrhage have been developed and used at USAISR to investigate the physiology of hemorrhage. Our investigators have designed unique methodologies to understand more fully the relationship between blood loss and BP in uncontrolled vs controlled hemorrhage. A major advantage of the use of animal models is the ability to make invasive physiologic measurements that otherwise cannot be easily attained in human subjects. In addition, the introduction of injury with hemorrhage using animals provides a unique capability to investigate the contribution of tissue trauma to the prediction of survivability. With the use of animals that are extensively instrumented with both invasive and noninvasive physiological monitoring sensors, numerous and various hemodynamic and metabolic variables can be measured before, during, and after recovery from moderate to severe hemorrhage. Most unique to the animal hemorrhage model is the ability to identify survival time as a clinical outcome for predicting the need for an LSI.

Trauma Patient Models. Although animal models offer numerous advantages to the study of mechanisms underlying hemorrhagic shock, the cardiovascular system and its regulatory components in animals do not necessarily function with responses identical to those observed in humans. Perhaps more importantly, most animal experiments require the use of anesthesia that can significantly alter autonomic reflex responses and eliminates the ability to assess significant human characteristics of mentation. It is therefore prudent that animal research be supplemented with a human clinical research arm that extends the applicability of experimental results.

The USAISR is continuing to develop a unique database that has been initiated in collaboration with Texas A&M University and the trauma center at the University of Texas Health Science Center at Houston, and will be extended to collaborations with trauma centers at Massachusetts General Hospital, Dartmouth Medical College, and in San Antonio. This part of the research plan will provide the unique opportunity to collect noninvasive, near continuous physiologic measurements on large numbers of injured patients. This database will be a large storage reservoir for physiologic data, clinical interventions, and outcome results of pre-hospital trauma patients. The ultimate aim of the research on trauma patients will be to collect data from the point of injury through the ambulance phase, into the emergency center, and ultimately through the operating room and intensive care unit. These data will be critical to the development of an accurate algorithm for remote triage on the battlefield because civilian trauma patients represent an operational human model for military casualties.[9] The USAISR is also in the unique position to expand the trauma patient database to include burn and trauma patients undergoing "elective" hemorrhage during surgery and recovery in the USAISR Trauma Division.

Human Hypovolemic Model. Data from trauma patients will be instrumental in providing etiology and military relevance for the understanding of hemorrhagic shock in humans.

However, the absence of physiological measurements at the time of injury until the moment that a medic arrives limits the ability to identify early predictors of clinical outcome. In an effort to extend the research capabilities to investigate mechanisms and early predictors of cardiovascular collapse during hemorrhage in humans, USAISR investigators have introduced a model designed to safely and noninvasively induce central hypovolemia in conscious human subjects, thereby eliciting hypotension and subsequent cardiovascular collapse like that resulting from hemorrhage.[10] This technology is based on the ability to redistribute blood away from the central circulation to the lower extremities with the use of lower body negative pressure (LBNP) (see Figure 2). Application of LBNP provides the capability of inducing cardiovascular and autonomic responses similar to those resulting from hemorrhage.[11,12] For example, Figure 3 shows a comparison of relationships between average reduction in central venous pressure and increased sympathetic nerve activity during hemorrhage (450 mL) and –10 mmHg LBNP in nine subjects.[11] It is clear that the relationships virtually mirror each other, suggesting that a blood loss to the central circulation of approximately one-half liter can be induced by each 10 to 15 mmHg LBNP. This assumption is based on well-established linear relationships between increasing LBNP and decreasing cardiac filling (central venous) pressure and stroke volume.[13-15] Figure 4 demonstrates the similarity in typical elevations in HR and reductions in cardiac output from a group of 10 pigs during actual hemorrhage compared with responses from a group of ten healthy test subjects to a graded LBNP protocol.[13,16] In the absence of effective resuscitative measures, compensatory reflex mechanisms fail to adequately compensate as levels of LBNP gradually increase, and a subsequent collapse of blood pressure regulation ensues with frank onset of severe hypotension (shock) and bradycardia similar to that reported in humans during severe hemorrhage.[14,17-19] The comparison of hemorrhage and LBNP data presented in Figures 3 and 4 demonstrate the similarity and potential for duplicating hemodynamic responses to actual hemorrhage with application of LBNP. Therefore, application of LBNP will provide a noninvasive method of investigating continuous and simultaneous cardiovascular responses and underlying mechanisms associated with hemorrhage in human subjects under conditions of controlled, experimentally-induced hypovolemic hypotension.

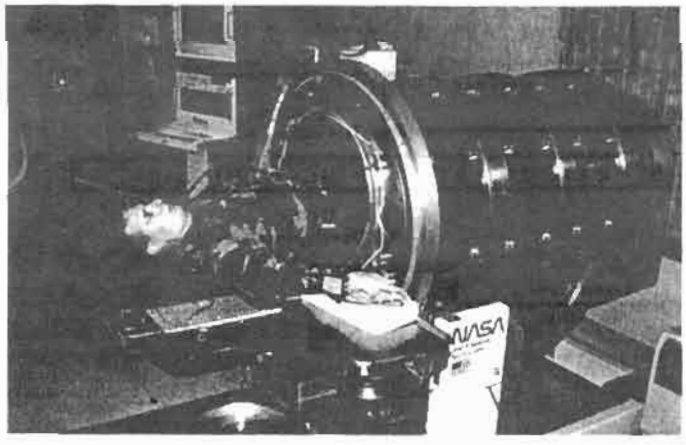

Fig 2. Subject placed in the LBNP device.

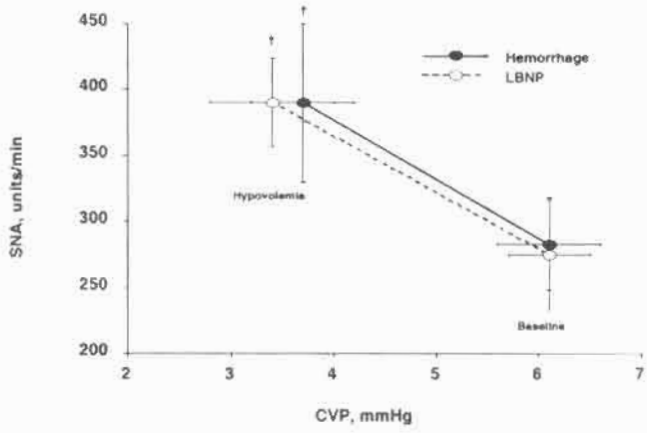

Fig 3. Comparison of relationships between central venous pressure and sympathetic nerve activity during -10 mmHg LBNP (open circles, broken lines) and 450 mL hemorrhage (closed circles, solid lines) in nine human subjects. Circles and lines represent mean ± SE values. † $P < 0.05$ compared with baseline. Data modified from Rea et al.[11]

Data Collection and Analysis

Figure 5 represents a diagrammatic summary of the cascade of activities that reflect the research plan for the Task Area for Advance Diagnosis for the Combat Medic in the Combat Casualty Care Research Program. First, integration of data from existing and ongoing animal and human experiments into a comprehensive trauma informatics database will significantly contribute to the development of new algorithms for automated remote triage of combat casualties. The primary strategy for development of a valid algorithm for early prediction of circulatory collapse will be focused on the simultaneous and continuous measurement of numerous physiological variables from human and animal subjects that comprise our hemorrhage trauma models. Measurements will be targeted to physiological responses associated with BP regulation. The resulting analog physiological signals will be collected and stored in a database that will allow for retrospective waveform analysis and data mining. Our analysis will focus on the hypothesis that it is possible to estimate mean time to circulatory collapse and the requirement for LSI from hemodynamic signals recorded in the USAISR database of hemorrhagic shock protocols. In addition to analysis of numerical responses, information content related to the morphology of specific physiological signals, their changes and variability over time, and their inter-relationships will be analyzed.

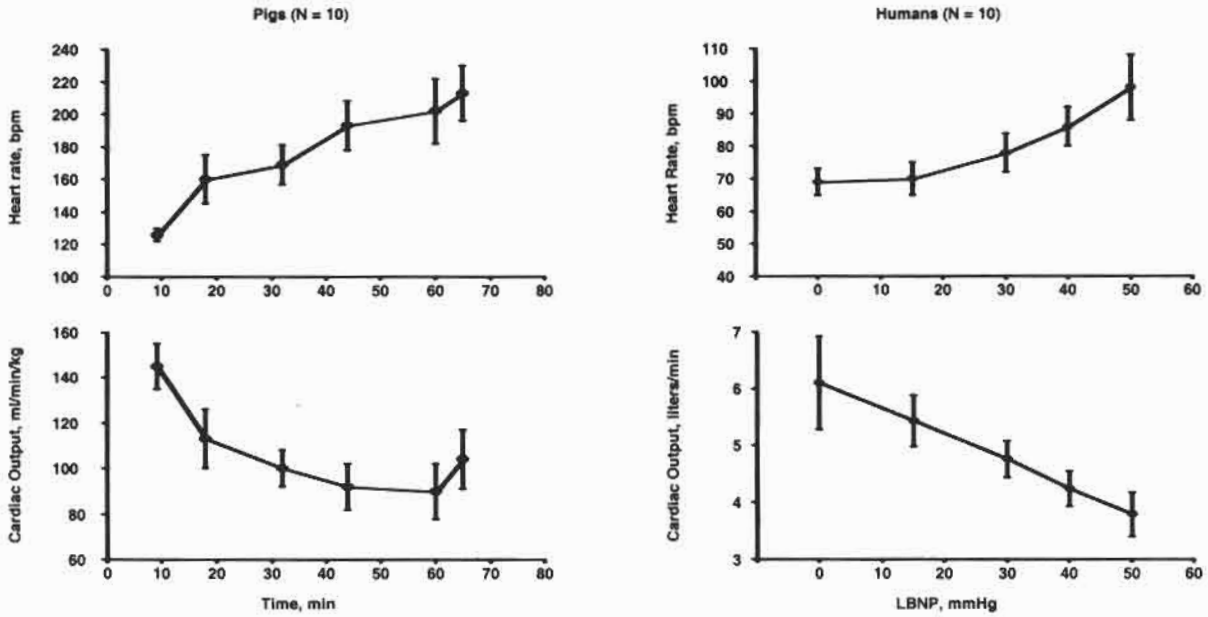

Fig 4. Comparison of elevated HR and reduced cardiac output during 65 min of hemorrhage in 10 pigs (left panels) and graded LBNP in 10 human subjects (right panels). Circles and lines represent mean ± SE values. Data modified from Hannon [16] and Convertino .[13]

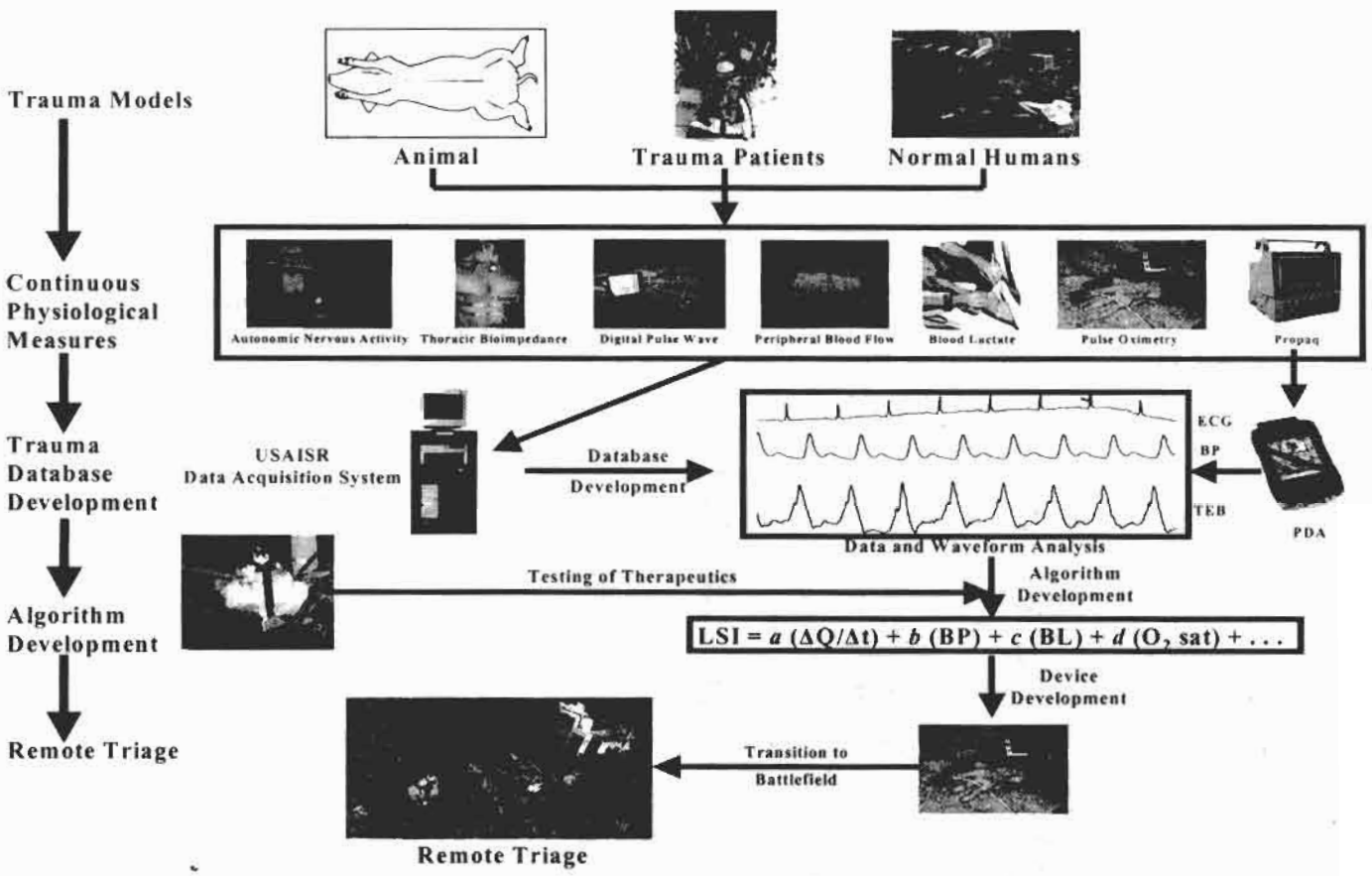

Fig 5. Flow diagram of research task area plan for advanced diagnosis and remote triage for the combat medic.

Algorithm Development

This research will apply the techniques of data mining to estimate mean time to circulatory collapse from hemodynamic, respiratory, and metabolic signs recorded in the USAISR database of hemorrhagic shock protocols. Data mining is exploration and analysis, by automatic or semiautomatic means, of large quantities of data in order to discover meaningful patterns and rules. Selected files containing data on human and animal models of hemorrhage and subsequent cardiovascular collapse will be analyzed. The independent variables will be the hemodynamic, neural, and metabolic parameters and the dependent variable will be time to circulatory collapse. Circulatory collapse will be defined by a precipitous fall in mean arterial pressure that becomes too low to maintain an adequate supply of cerebral blood flow or mental function. Independent variables will include (but are not limited to) HR, BP, stroke volume, cardiac output, peripheral blood flow, cerebral artery blood flow, arterial O_2 saturation, blood gases and metabolites, autonomic nervous activities, sublingual CO_2, electrocardiogram waveform, peripheral pulse waveform, vascular volume status, mentation, and clinical outcome. Significant early predictor(s) of failure (cardiovascular collapse in human subjects and mortality in animals) will be identified using multiple logistic regression statistics. Methods will include correlation coefficients, multiple logistic regression, Reed-Muench analysis, Kaplan-Meier survival analysis, cluster analysis, and discriminate analysis.

Device Development and Transition for Battlefield Use

The resulting algorithm for early prediction of cardiovascular collapse that evolves from the Research Task Area will inherently identify the specificity and frequency of physiologic measures required in order to provide the most effective casualty care and remote triage. The resulting physiologic measures extracted from the remote triage algorithm can then be used to direct decisions regarding development or identification of medical monitoring devices or technologies that can be worn by the soldier. For example, a small computer that includes the algorithm could be part of the monitoring system worn by the soldier. A personal digital assistant device carried by the battlefield medic would provide a simple visual code (green, yellow, red) of the soldier's medical status that can be transmitted via global-positioning satellites. If integrated into the proposed Warrior Physiological Status Monitor, the Advanced Medical Monitoring Device could reduce combat mortalities by enabling combat medics to: (1) commence triage within moments after a soldier is wounded; (2) receive more accurate information of wound severity and progression to shock; and, (3) optimize available treatment and evacuation. Finally, since the killed in action rate for battlefield medics has been as high as double that of infantrymen, the advanced diagnosis system could be instrumental in reducing battlefield mortality of medics by providing early identification of dead soldiers.

Summary

With the use of experimental protocols that utilize human and animal trauma models of central hypovolemia leading to cardiovascular collapse, the goal of the Task Area for Advance Diagnosis and Triage is to provide an automated capability for remote trauma triage on the battlefield. This goal will be accomplished through an extensive series of research projects designed to provide continuous data acquisition of numerous noninvasive physiological signals (measurements) associated with BP regulation. With the use of data mining, neural networks, multivariate logistic regression analysis, and decision tree analysis, an algorithm predictive of clinical outcome and the need for lifesaving procedures can be generated from the resulting physiologic database. Not only will these data be utilized to direct the future of combat casualty care monitoring systems, but they will facilitate rational decisions concerning bandwidth requirements and, perhaps more importantly, what physiologic measurements are truly important predictors of outcome and LSI. This research approach will provide the first ***data-driven*** answer for ***remote triage*** for the ***Objective Force Warrior***. The resulting algorithm will enhance the diagnosis and acute treatment of hemorrhagic wounds. It is in this manner that our approach will produce an enhanced human capability for advanced diagnosis and remote triage of combat casualties and will ultimately be able to improve survivability of combat casualties.

References

1. Bellamy RF. The causes of death in conventional land warfare: Implications for combat casualty care research. *Mil Med.* 1984;149:55-62.

2. Zajtchuk R, Sullivan GR. Battlefield trauma care: focus on advanced technology. *Mil Med.* 1995;160:1-7.

3. Orlinsky M, Shoemaker W, Reis ED, Kerstein MD. Current controversies in shock and resuscitation. *Surg Clin North Am.* 2001;81:1217-1262.

4. Wo CJ, Shoemaker WC, Appel PL, et al. Unreliability of blood pressure and heart rate to evaluate cardiac output in emergency resuscitation and critical illness. *Crit Care Med.* 1993;21:218-223.

5. Bland R, Shoemaker WC, Shabot MM. Physiologic monitoring goals for the critically ill patient. *Surg Gyn Obst.* 1978;147:833.

6. Shoemaker WC. Diagnosis and therapy of shock and circulatory dysfunction. In: *Textbook of Critical Care*, 4th ed. Shoemaker WC, Grenvik A, Ayers SM, eds. WB Saunders, Philadelphia; 2000: p 92-113.

7. Shoemaker WC, Appel PL, Kram HB, et al. Prospective trial of supranormal values of survivors as therapeutic goals in high risk surgical patients. *Chest.* 1988;94:1176-1186.

8. Shoemaker WC, Appel PL, Kram HB. Role of oxygen debt in the development of organ failure, sepsis, and death in high risk surgical patients. *Chest.* 1992;102:208-215.

9. Schreiber MA, Holcomb JB, Conaway CW, Campbell KD, Wall M, Mattox KL. Military trauma training performed in a civilian trauma center. *J Surg Res.* 2002;104:8-14.

10. Convertino VA. Mechanisms of blood pressure regulation that differ in men repeatedly exposed to high-G acceleration. *Am J Physiol.* 2001;280: R947-R958.

11. Rea RF, Hamdan M, Clary MP, Randels MJ, Dayton PJ, Strauss RG. Comparison of muscle sympathetic responses to hemorrhage and lower body negative pressure. *J Appl Physiol.* 1991; 70:1401-1405.

12. Wolthuis RA, Bergman SA, Nicogossian AE. Physiological effects of locally applied reduced pressure in man. *Physiol Rev.* 1974;54:566-595.

13. Convertino VA. Gender differences in autonomic functions associated with blood pressure regulation. *Am J Physiol.* 1998;275:R1909-R1920.

14. Convertino VA. Lower body negative pressure as a tool for research in aerospace physiology and military medicine. *J Grav Physiol.* 2001;8:1-14.

15. Convertino VA, Doerr DF, Ludwig DA, Vernikos J. Effect of simulated microgravity on cardiopulmonary baroreflex control of forearm vascular resistance. *Am J Physiol.* 1994;266:R1962-R1969.

16. Hannon, JP. Hemorrhage and Hemorrhagic Shock in Swine: A Review. In: *Swine as Models in Biomedical Research.* M.M. Swindle, ed. Ames: Iowa State University Press; 1992: p 197-245.

17. Barcroft H, Edholm OG, McMichael J, Sharpey-Schafer. Post-hemorrhagic fainting. *Lancet.* 1944;I:489-491.

18. Foex BA. Systemic responses to trauma. *Brit Med Bull.* 1999;55:726-743.

19. Secher NH, Jensen KS, Werner C, Warberg J, Bie P. Bradycardia during severe but reversible hypovolemic shock in man. *Circ Shock.* 1984;14:267-274.

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Research on Tourniquet-Related Injury for Combat Casualty Care

Thomas J. Walters, PhD†

The tourniquet has been used for over 300 years for effective hemorrhage control during surgery and trauma. However, tourniquets are far from benign, causing a host of complications collectively known as tourniquet injury. A tremendous body of clinical experience and scientific research has resulted in principles of safe use and advances in tourniquet design, minimizing tourniquet injury under clinical conditions. Unfortunately, battlefield conditions preclude adherence to these safe principles and the use of surgical tourniquets. The U.S. Army Institute of Surgical Research (ISR) has developed an integrated program designed to address the unique nature of tourniquet use under combat conditions with the goal of increasing the rate of limb salvage and saving lives.

Introduction

Since the tourniquet was introduced in 1674 on the battlefield by the French military surgeon, Moral, it has been routinely used to control bleeding during surgery or following extremity trauma involving severe vascular damage. While properly applied tourniquets are extremely effective in controlling hemorrhage, their use is far from benign. Tourniquet related injury consists of compression injury to the underlying skin, nerve, and muscle, as well as ischemia/reperfusion (I/R) injury to the underlying and distal muscle and nerve.[1,2] When tourniquets applied for long durations are removed, a severe systemic inflammatory response leading to damage to remote organs can take place, in some cases resulting in fatality.[3] This understanding has lead to clinical practices and advances in tourniquet design that have minimized the risk of these complications during surgery. Specifically, minimizing tourniquet application duration to less than 2 hours and the use of wide, pneumatic tourniquets that minimize tissue compression, have lead to safe and practically complication free use.[1]

Unfortunately, the circumstances that dictate the use of tourniquets on the battlefield typically exclude compliance with safety principles and tactical constraints often violate the 2-hour safe period. The duration of trauma tourniquet application is usually controlled by the length of time it takes to evacuate the soldier to a far-forward medical treatment facility for definitive vascular repair, a delay that often exceeds 2 hours. While it is well recognized that extended tourniquet application often results in the loss of muscle function or limb amputation, it has been generally accepted that the priority is "life over limb."

Design is another major distinction between surgical and trauma tourniquets. The wide, pneumatic tourniquets popular in surgery today are not practical on the battlefield. Specifically, they are too large to carry. Because life threatening extremity arterial wounds are often near the groin or auxiliary regions, the width of a surgical tourniquet may preclude effective placement for these wounds. Finally, concerns over the inherent propensity of pneumatic bladders to leak have lead to their dismissal as impractical on the battlefield. As a result, the military has seen virtually no advancement in reducing tourniquet injury. Even the newly fielded one-handed tourniquet (see Ryan this issue), while effective for hemorrhage control, does not resolve the tourniquet related injury observed on the battlefield over 300 years ago.

It is the goal of our program to advance tourniquet design and to optimize limb salvage by integrating the relevant scientific, clinical, and military medical literature supported by our own laboratory studies to produce: (1) tourniquet guidelines; (2) medical treatments; and (3) new tourniquet designs to optimize limb salvage.

Military Tourniquet Experience

Although the scientific literature contains little research on the consequences of tourniquets during trauma, the pre-Vietnam military medical literature contains a wealth of relevant information based on thousands of cases involving tourniquet application. The majority of these reports document cases during WWII.[4] This information constitutes a resource virtually unknown to modern day military medical personnel, unavailable in medical reference databases. Currently, a major effort is underway to unearth this literature for future publication in the form of a review article. In addition, this material will be combined with a review of current experimental data to create a knowledge base for the generation of tourniquet guidelines for optimizing limb salvage by a consensus panel at the Advanced Technology Applications for Combat Casualty Care Conference in August 2003.

Small Animal Studies

Characterizing Tourniquet Injury In An Animal Model.

Research at the ISR was initiated 2 years ago with the development of a rat model of tourniquet injury. Using a pneumatic tourniquet system, we have explored a range of durations of tourniquet application for which we have assessed animals at 2 hours, 2 days, or 2 weeks following tourniquet release. These time points were chosen as they characterize the acute injury (2 hours), the peak of the injury process (2 days), and the intermediate stage of muscle recovery and regeneration (2 weeks). Muscle injury is determined primarily by examining muscle function using an in situ preparation, as well as standard histology and vital staining. Muscle edema and atrophy are determined using wet weights and wet to dry weight ratios.

The most obvious response to tourniquet release is profound edema (Figure 1). The affected limb is paralyzed, with no response to painful stimuli for at least 2 days. In situ, muscles do not respond to electrical stimulation of the motor nerve. However, direct stimulation of the muscle does elicit force production, although it is well below that produced by the corresponding contralateral muscle. Taken together, these observations indicate both nerve and muscle injury (Figure 2). At day 14, peak force production is similar regardless of whether the muscle is stimulated directly or via the motor; however, it is significantly reduced compared with the contralateral control muscle.[5]

Fig 1. Rat model. Representative photograph demonstrating the extreme level of edema 2 days after 4 hours of tourniquet application. The lack of toe spreading in the affected left limb indicates neural injury.

The magnitude of the injury depends on the muscle examined. The plantaris, a predominantly fast-twitch (type II) muscle is significantly more vulnerable to tourniquet injury than the predominantly slow-twitch (type I) soleus muscle (Figure 3).[5,6] A hallmark of aerobic training is a shift in the metabolic profile to that characterized by type I muscle fibers (for examples, high mitochondrial content and capillary density).[7] Thus, fitness level prior to injury may be an important mediator in determining the extent of tourniquet injury. Regardless, a better understanding of the specific reasons for these differences should help in the development of treatments that can reduce the magnitude of the injury and hasten recovery.

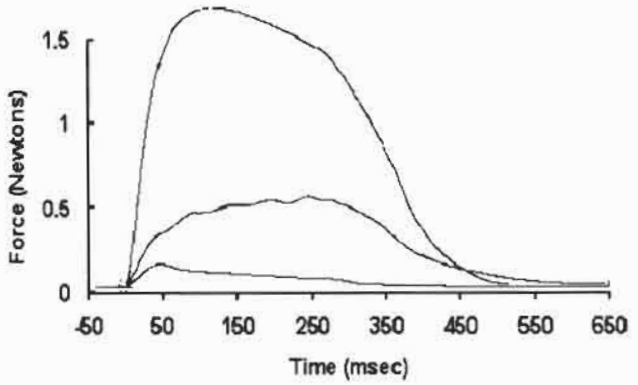

Fig. 2 Force traces. Stimulation of the motor nerve resulted in little force production (A). Direct stimulation of the muscle resulted in greater force (B), demonstrating injury to motor nerve. Regardless, force is significantly reduced compared to the contralateral control (C). This pattern occurred in all animals tested on day 2 independent of muscle (soleus or plantaris) or tourniquet duration (2 or 4 hours).

Effect of Hemorrhage Induced Hypotension on Tourniquet Injury. Clinically, tourniquets are used to create a bloodless surgical field. In contrast, a trauma tourniquet is preceded by severe hemorrhage. We are currently addressing the question of whether hemorrhage induced hypotension impacts the extent of tourniquet injury. In these studies, animals undergo a hemorrhage of approximately 35%-40% of their total blood volume, followed immediately by tourniquet application for 4 hours. These animals are then compared to appropriate groups that have undergone a sham hemorrhage. These studies are currently in progress and will be of importance in determining the injury pattern from tourniquet use after trauma induced blood loss.

Remote Injury. Tourniquets are known to cause injury to remote organs.[3,8] The extent of the injury is related to the duration of tourniquet application. This is of minimal clinical concern in civilian surgery, however, the extended period of time between tourniquet application on the battlefield and removal of the tourniquet at far-forward medical treatment, make it a significant concern for combat casualty care. We have done preliminary studies of the damage to all major organ systems following 3 hours of tourniquet application. 3-nitrotyrosine (3-NT), a biochemical maker of cellular stress, was significantly elevated in the lung and liver following 3 hours of

tourniquet application in our rat model (Figure 4).[9] These responses were rather modest, however, this is not unexpected as the magnitude of systemic responses may be significantly affected by the mass of the directly injured tissue (the rather modest responses observed may be a function of the relatively small muscle mass involved within the rat model). Furthermore, while military applications dictate tourniquet use for hemorrhage control, hemorrhage per se has been associated with increases in nitrosative stress. We are, therefore, currently investigating the effects of both the mass of the injured muscle, as well as tourniquet application in combination with hemorrhage, on systemic nitrosative stress. In addition to nitrosative stress, ongoing studies are examining a number of indicators to quantify the systemic inflammatory response.

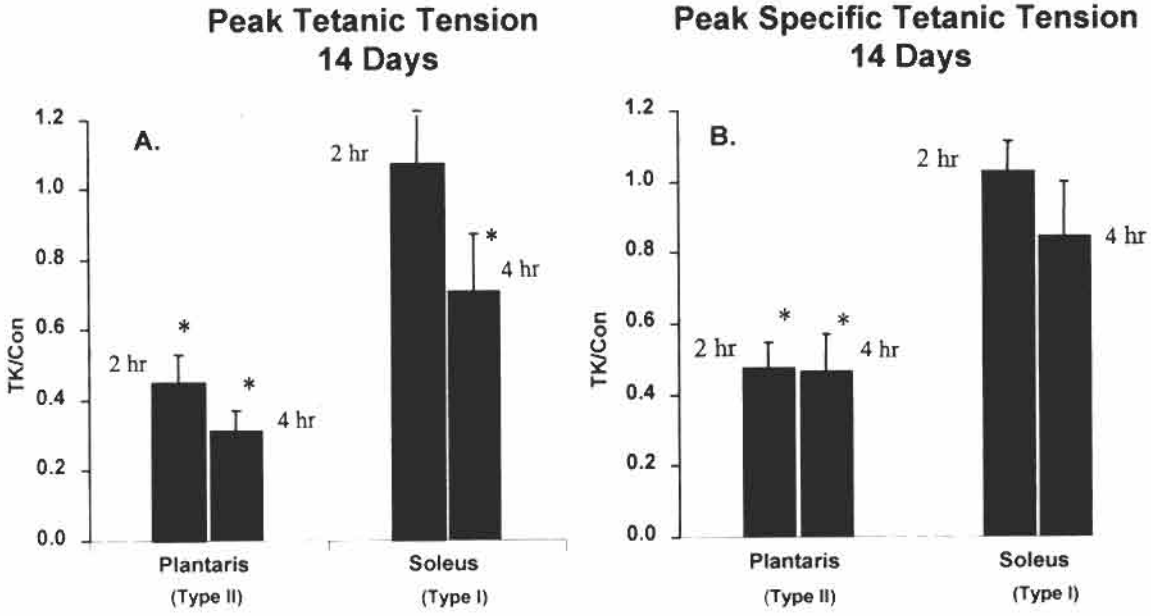

Fig 3. (A) Ratio of tetanic tension of treatment/contralateral control muscles. Significant reductions took place for all muscles and treatments with the exception of the soleus at 2 hours tourniquet. (B) Ratio of normalized (muscle wt) tetanic tension of treatment/ contralateral control muscles. Significant reductions took place only in the plantaris, indicating an increase in noncontractile elements, probably fibrotic tissue. In contrast, the reduction in force for the soleus reflects atrophy and/or fiber loss. Different from contralateral control (P< 0.05).*

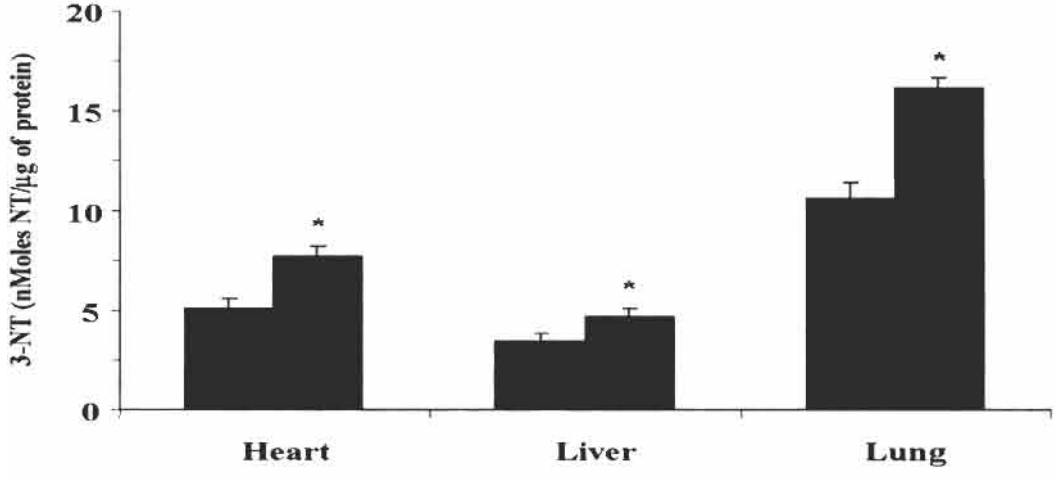

*Fig 4. 3-NT levels in heart, liver, and lung following 3 hours of tourniquet application and 2 hours of reperfusion. *P ≤ 0.05 compared with control value. 3-NT is an indicator of tissue damage caused by protein nitration.*

Gene Expression Profiles. The reduction of tourniquet injury through the development of pharmacological interventions requires an understanding of the response of muscle to both the ischemic and reperfusion phases of injury. The most efficient method for assessing the response of a cell or tissue to injury or a drug is with gene expression analysis with cDNA microarrays. The gene expression profile of skeletal muscle in response to I/R is currently unknown so we are using this technique to characterize I/R injury in skeletal muscle using our rat tourniquet model. An understanding of the genetic response to both ischemia and reperfusion may lead to pharmacological interventions and therapies that can address both components of this injury, leading to greater tissue salvage and ultimately saving limbs.

Large Animal Studies

Reducing Duration of Tourniquet Use. Many of the injurious effects of tourniquets cannot be avoided. Regardless of advances in tourniquet engineering and treatments to reduce the injury process, biophysical limits will always exist. However, recent development and fielded hemostatic agents and polymeric wound dressings can be used in conjunction with a tourniquet to reduce the required duration of tourniquet application. This scenario involves initial, prompt application of a tourniquet to a severely bleeding extremity by the injured soldier or a buddy. When the tactical situation allows, a medic would then apply an appropriate wound dressing, release (not remove) the tourniquet and then observe for effectiveness of the wound treatment. If hemorrhage is not adequately controlled, the tourniquet would again be tightened. Successful control of hemorrhage by a wound dressing would obviously reduce tourniquet injury, which results from physical compression. Additionally, it would reduce I/R by allowing reperfusion of the limb by the remaining patent collateral circulation. Together these factors would greatly increase the chances of limb salvage.

Our lab has developed a fatal extremity wound model using the anesthetized goat to test wound treatments according to this scenario. A tourniquet is initially used to control hemorrhage following wounding by creation of a vascular defect. The candidate wound dressing is then applied. The tourniquet is then released and the animal is observed for 1 hour. Successfully treated animals survive through 1 hour while treatment failures and controls (no dressing applied) exsanguinate. Figure 5 demonstrates the severity of the injury as evidenced by the rapid fall in mean arterial pressure following wounding and the release of the tourniquet. To date, only one (unsuccessful) candidate has been tested using this model. However, future plans include the testing of a number of products that may prove efficacious.

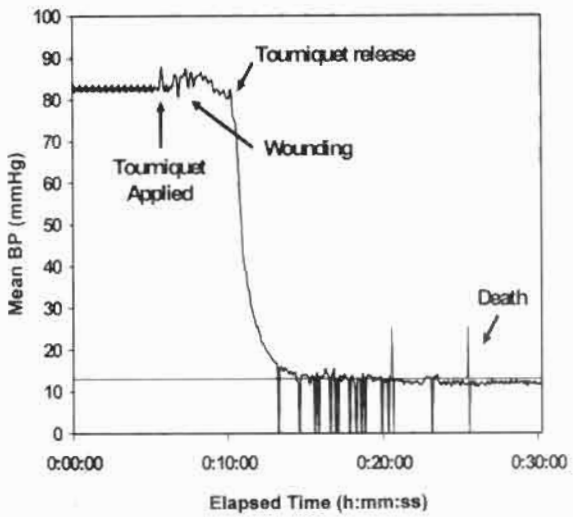

Fig 5. A representative trace of the mean arterial pressure before and after inflicting a fatal arterial injury to the femoral artery in a goat control animal. The operational definition of death is a mean arterial pressure below 13 mmHg for 5 minutes, which occurred in this example in approximately 12 minutes from the time of tourniquet release. The severity of the injury makes it ideal for the rigorous testing required for confident product testing

Product Development

Advanced Tourniquet Design. Doctor Jan Gooch, a current National Research Council Senior Fellow at the Institute, has focused on designing and engineering the initial models of the next generation of military trauma tourniquets. Ongoing interaction with Special Operation Force medics assures the design parameters meet the requisite flexibility and cube constraints required for the battlefield. As discussed above, concerns regarding inherent leaking and the bulky nature of pneumatic orthopedic tourniquets have kept these devices from being fielded for combat use. However, the appreciation of the superiority of a pneumatic design was recognized by far-forward military personal over 60 years ago.[10] Considering all of these concerns, Dr Gooch's current prototype is a self-contained narrower version of an orthopedic tourniquet. By employing a self-inflating system equipped with a servo system to monitor and maintain a prescribed pressure, the system averts the problem of leaking. Additionally, in the event of a catastrophic leak, the system can be used in the manner of a traditional strap-and-buckle tourniquet. Formal testing of these systems on phantoms, followed by human subjects, is planned for the next year.

Concluding Remarks

We have presented a description of our program that takes an integrated approach to reducing tourniquet related injury.

Scientific research and military experience will produce new treatments, procedures, guidelines, and devices aimed at a single goal, to change the axiom "saving life over limb" to "saving life and limb."

References

1. Love BR. The tourniquet. *Aust N Z J Surg*. 1978;48(1):66-70.

2. Pedowitz RA. Tourniquet-induced neuromuscular injury. A recent review of rabbit and clinical experiments. *Acta Orthop Scand*. 1991;245(suppl):1-33.

3. Seekamp A, Ward PA. Ischemia-reperfusion injury. *Agents Actions*. 1993;41(suppl):137-52.

4. Wolff LH, Adkins TF. Tourniquet problems in War Injuries. The Bulletin of the U.S. Army Medical Department. 1945; 87:77-84.

5. Walters TJ. Tourniquet Injury: Alteration in Muscle Function. Advanced Technology Applications for Combat Casualty Care. St Petersburg Beach, FL; September 2002.

6. Walters TJ. Skeletal Muscle Fiber Type Impacts Vulnerability to Tourniquet-Induced Injury. Federation of American Societies for Experimental Biology (Biology 2003) at San Diego, CA; 2003.

7. Holloszy JO, Coyle EF. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol*. 1984;56(4):831-8.

8. Blaisdell FW. The pathophysiology of skeletal muscle ischemia and the reperfusion syndrome: a review. *Cardiovasc Surg*. 2002;10(6):620-30.

9. Ryan KL, Fernandez R, Walters TJ. Systemic Nitrosative Stress And Cytokine Levels Following Hindlimb I/R. Federation of American Societies for Experimental Biology (Biology 2003) at San Diego, CA, 2003.

10. Bailey H. Surgery of Modern Warfare. Edinburgh, England, E&S. Livingstone, 1941, 273-279.

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Bone and Soft Tissue Trauma Research at the ISR

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Introduction

Since its establishment in 1943, the Institute of Surgical Research (ISR) has conducted research focused on improving the surgical care given to soldiers. Just as our predecessors addressed the unacceptably high impact of thermal injury on combat casualties, the Bone and Soft Tissue Research Team focuses research on combat casualties by examining the epidemiology of combat wounds to identify needed improvements in combat casualty care. This article provides an overview of the bone and soft tissue trauma research currently being conducted at the ISR and introduces the available combat casualty data from recent conflicts that were used to identify areas where research can achieve maximum impact on the morbidity and mortality of combat casualties.

Combat Casualty Statistics

Given that the Vietnam War ended over a quarter century ago, we sought data from more recent conflicts on which to base our research efforts. While we have been fortunate to have suffered relatively few combat casualties at the end of the 20th century, Operation Just Cause (Panama), Operation Desert Storm (Kuwait and Iraq), and the Battle of the Black Sea (Somalia) offered the opportunity to investigate more recent patterns of combat injury. Fortunately, the patterns of injury were documented both during and after each of these conflicts, as had been done in Vietnam by the Wound Data and Munitions Effectiveness Team.

Despite the great disparity in missions, environments, enemy, weaponry, and the units engaged, the distribution of wounds was surprisingly consistent in these three conflicts (Table and Figure 1). In each conflict, extremity wounds predominated, constituting between 70-75% of all wounds. Another interesting finding from the data collected following Operation Just Cause is the preponderance of minor to moderate wounds in combat casualties as indicated by injury severity scores (Figure 2), a finding consistent with a similar analysis of casualties from Vietnam. With this new appreciation of the importance of extremity wounds in general, and wounds of mild to moderate severity in particular, we set out to identify medical interventions which target these types of wounds.

Injury Location	OJC	ODS	Somalia	Weighted Average
Extremity	70%	71%	75%	71%
Thorax	9%	4%	7%	6%
Head/Neck	9%	15%	14%	13%
Pelvis	4%	2%	1%	3%
Abdomen	4%	4%	3%	4%
Eye	2%	2%	0%	2%
Spine	1%	1%	0%	1%

Table. Anatomical Distribution of Injury as a Percentage of Total Number of Wounds in Observed in Soldiers Wounded in Action During Operation Just Cause, Operation Desert Storm, and the Battle of the Black Sea (Somalia)

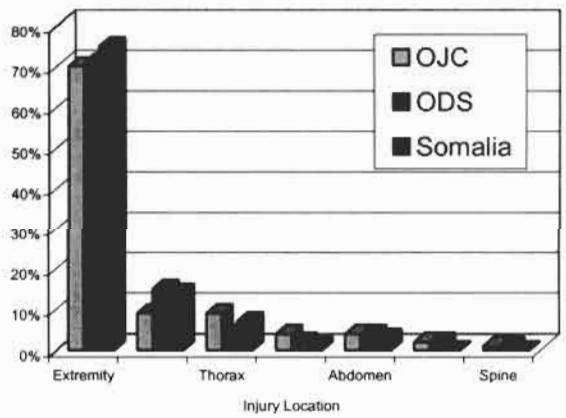

Fig 1. Anatomical Distribution of wounds which were recorded for WIA in Operation Just Cause, Operation Desert Storm, and Somalia. Data were compiled from McBride, et al (Operation Just Cause), Uhorchak et al. (Operation Desert Storm) and Mabry, et al (Somalia).[5,7,8] The patterns of anatomical distribution of injury are highly consistent, and highlight that extremity wounds cause a greater number of casualties than all other wounds combined.

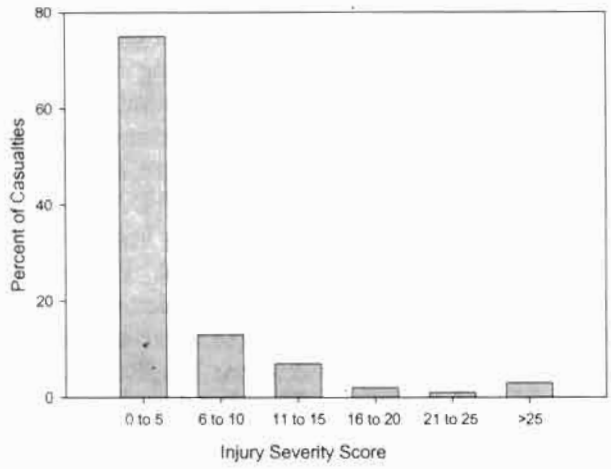

Fig 2. Injury severity scores for casualties from Operation Just Cause (Panama).

Battlefield Treatment of Fractures

Of the three-fourth of combat wounds that are to the extremities, a large fraction of these injuries include trauma to bone. In fact, fractures constituted 28% of the combined 941 injuries documented for Desert Storm, Just Cause, and Somalia. Not only are these an extremely common injury, fractures in combat casualties result in unusually high morbidity due to high rates of bone loss, mal-union, and osteomyelitis. To reduce the negative impact of fractures, we are developing products that start at the buddy/medic level (noninvasive pelvic band for fracture stabilization and improved splints and casts) and that continue through all echelons of treatment to definitive care (antimicrobial coated external fixator pins and antimicrobial bone graft substitute).

Pelvic Fracture Stabilizer. While pelvic fractures constitute a relatively small proportion of the fractures on the battlefield, they do occur. These fractures are caused by falls during airborne and fast-rope insertion, as well as miscellaneous accidents. In order to improve our ability to stabilize these casualties during evacuation and the initial phases of diagnosis and treatment in echelon II medical treatment facilities, we have conducted an analysis of commercially available pelvic compression bands. Cadaveric studies conducted at the ISR and by academia have documented the utility of these devices in stabilizing the pelvic ring, and reducing the volume of the pelvic cavity, which may aid in hemostasis. In addition, clinical experience suggests that improved stability during transport and manipulation of the patient for medical evaluation significantly reduces pain. These devices are lightweight and inexpensive, and much easier to use than improvised devices and invasive fixators. As a result, we anticipate medical evacuation vehicles will carry pelvic bands in the future.

Improved Splint/Cast. Although casts are highly effective treatment for fractures, plaster of paris requires water and is high in both cube and weight, making it a less than ideal substance in far-forward medical treatment facilities. Given the great advances in materials science in recent decades, we hypothesized that a polymer could be identified and engineered that would replicate the mechanical properties of plaster and gauze casts while greatly reducing weight. These materials are currently under development through several partnerships with both academia and industry. One promising candidate system, a composite Kevlar mesh/polyurethane epoxy, achieves high strength and rigidity, and is extremely lightweight. This composite system can be formed to an extremity as it is pliable before the epoxy completes curing then rapidly stiffens and provides mechanical stability to a fractured limb. Although the impetus for the development of this product is the burdensome weight of plaster casts, we also anticipate that these improved devices may serve as splints, particularly in Special Operations units which operate without easy access to evacuation to an echelon II facility.

Another promising line of research seeks to develop a splint that can off-load the lower extremities. Figure 3 illustrates the impact of minor extremity wounds on units in combat. In this photograph from the recent war in Iraq, a single wound to a lower extremity required three uninjured personnel to aid in evacuation. A device that would allow a soldier to remain ambulatory after injury could have a significant impact on unit effectiveness. We do not anticipate returning the wounded soldier to full function without further care, however, merely freeing other soldiers from the need to carry the casualty would significantly impact unit combat effectiveness. In addition, the casualty may be able to continue to contribute to mission success by performing limited duties such as manning a defensive position.

Fig 3. Although not life threatening, wounds to the lower extremities can have significant impacts on combat units as a wounded soldier who is unable to ambulate requires 2-4 uninjured solders to be removed from the fight in order to aid in evacuating the casualty. This photo shows three Marines carrying a lower extremity casualty during the early days of the second Gulf War.

Antimicrobial Coated External Fixator Pins. Once a casualty with an open fracture reaches a treatment facility with surgical capability, external fixation is often used to provide mechanical stability while the soft tissue wound heals. Due to the combined effects of higher inoculum and longer delays between injury and initial surgical wound care (irrigation and debridement), external fixators are often used for extended periods. One possible outcome of this extended period of external fixation is pin tract infection, which can lead to pin loosening (loss of structural stability), osteomyelitis, and delays in conversion to internal fixation.[1,2] In order to combat this problem, the ISR has developed and tested several prototype antimicrobial coated external fixator pins. Of those tested, standard stainless steel or titanium pins coated with a combination of hydroxyapatite (the calcium-containing mineral found in bone) and lipid stabilized chlorhexidine (a potent antimicrobial) (Figure 4) proved the most effective, with an 80% reduction in the rate of pin infection in a large animal model of intentionally contaminated pins.[3,4] These coated pins have been nominated for advanced development, and investigators at the ISR are currently working with U.S. Army Medical Materiel Development Activity and industry partners to speed the fielding of this promising product.

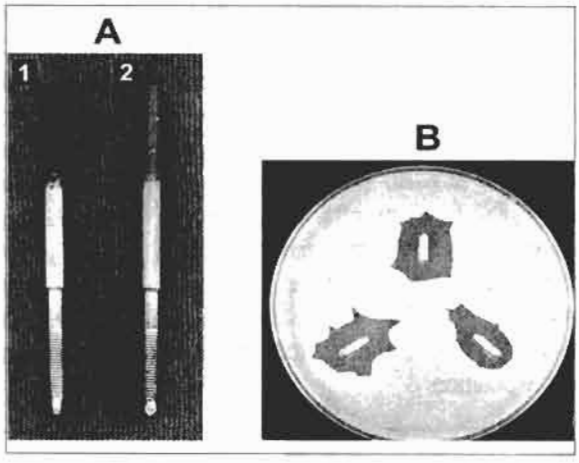

Fig 4. Antimicrobial coated external fixator pins. The pins shown in panel A are commercially available stainless steel (1) and titanium (2) self-tapping external fixator pins that have been coated with lipid stabilized chlorhexidine and hydroxyapatite in order to improve the stability of the interface between the bone and the pin and reduce the incidence of pin tract infection related complications. Panel B shows sections of coated pin placed onto a Petri dish seeded with Staphylococcus aureus bacteria. The clear region surrounding each pin section indicates that bacteria were unable to grow around these pins and is indicative of the effective local antibacterial action of the coated pins.

Antimicrobial Bone Graft Substitute. Grossly contaminated open fractures are commonly treated with prophylactic local antibiotics. Local delivery of antibiotics involves the implantation of antibiotic impregnated cement beads that are fabricated in the operating room by combining polymethylmethacrylate and an antibiotic, and hand forming this paste into small spheres. These beads elute antibiotic for a period of 2 to 6 weeks, then become nothing more than a foreign body, which require surgical removal. Thus the currently available treatment for a grossly contaminated open fracture requires multiple surgeries. Furthermore, these cement beads are not Food and Drug Administration (FDA) approved, do not enhance healing, may in fact negatively impact healing, and delay autologous bone graft placement. The repeated implantation and removal of antimicrobial polymethylmethacrylate beads require multiple surgeries resulting in extended hospital stays and convalescence. The ISR is currently conducting research to test a single product that can serve both as the antimicrobial implant to sanitize the wound as well as an osteo-inductive matrix to reduce the number of surgeries to a single trip to the operating room, and thereby speed wound healing. Our research program to develop and test antimicrobial bone graft substitutes is the subject of the article by Beardmore et al in this issue. We believe that improved splints, noninvasive pelvic stabilizers, and antimicrobial external fixator pins can improve combat casualty care beginning at the level of self and buddy aid, through combat medic care and stabilization, to repair in surgery-capable treatment facilities.

Soft Tissue Trauma Care

Soft tissue trauma that occurs as the result of combat wounds is a diverse category which includes the entire spectrum of injuries from minor to severe. While it is tempting to ignore the need to treat minor wounds in favor of focusing on wounds that are life-threatening, the statistics cited above highlight the need to address the entire spectrum of wounds. Although minor wounds have less impact on the individual soldier, they are by far the most common, and even moderate decrements in soldier health can have severe impacts on unit capability when multiplied by their high incidence rate. A minor wound that may require only minimal care is at risk for infection. Data from Somalia as well as from British casualties in the Falkland Islands Campaign show that approximately 15% to 20% of combat wounds result in infection.[5,6] Infected wounds result in increased morbidity and mortality when compared to similar wounds that are not infected, and lead to longer hospitalization. ISR has focused soft tissue trauma research on preventing infectious complications in mild, moderate, and severe wounds, improving wound care for soft tissue wounds which require treatment in a forward medical treatment facility, and reducing the rate of limb loss due to tourniquet application.

Prevention and Treatment of Wound Infection. In order to reduce the rate of both soft and hard tissue infection, the ISR initiated a program to push antibiotic therapy into the pre-hospital phase of medical care. For conventional forces, antibiotics are currently unavailable in the field. Although some

Special Force medics carry and administer antibiotics, current drugs require intravenous access, which can be problematic in the far-forward environment. A review of the relevant literature showed that the pharmaceutical industry has developed promising oral antibiotics with low toxicity, excellent bioavailability, and long half-lives. In collaboration with the U.S. Special Operations Command, the ISR convened a panel of military and civilian subject matter experts, representing Military Medicine, Infectious Disease, Surgery, Pharmacology, and Microbiology to review currently available, FDA approved antibiotics for self-aid. The consensus of the panel was that gatifloxacin (Tequin®) is the most appropriate drug for self-administration any time a combat wound results in a break in the skin. This recommendation was made to the AMEDD Center and School Directorate of Doctrine Development and is currently being implemented.

Advanced Wound Dressings. The gauze field first aid dressing and recently developed and fielded hemostatic dressings (see article in this issue by Kheirabadi, et al) provide wounded soldiers with excellent tools for the control of hemorrhage. However, these dressings are not practical for the protection of mild to moderate wounds. Interviews with combat medics indicate that the standard field dressings will often slide distally if a casualty resumes normal activities. The failure to properly protect mild to moderate wounds from both bacterial contamination and further mechanical damage can be the source of high wound infection rates and an unacceptable degradation of individual performance. In order to provide a dressing for these wounds, we have initiated research on dressings that can be sprayed, painted, or dusted on to soft tissue wounds to provide a barrier to contamination and abrasion. Work to date has identified an extremely promising polymer product developed by an industry partner, and modified to meet the need for a combat wound dressing. In order to assess the efficacy of this dressing, we have conducted tests utilizing an animal model of a contaminated soft tissue wound. This product outperformed other candidate products, and has the potential to serve as paint on protection that will reduce pain and wound infection, while speeding healing and maintaining maximum individual performance, thus reducing the need for evacuation. A clinical trial is currently in progress as an initial step toward transition of this product to advanced development and fielding.

Wound Irrigation and Tissue Viability. For a typical traumatic wound, devitalized tissue is debrided and wounds are irrigated with what is usually described as "copious" or "adequate" volumes of sterile saline. While inexpensive in a fixed treatment facility, sterile saline can be an extreme logistical load for the deployable treatment facility, especially if current dogma is followed and 8 to 12 liters of saline are used to irrigate each wound. In order to minimize the weight and cube of fluid consumed for initial wound care, investigators at the ISR are progressing on two fronts.

First, we are attempting to reduce volume of fluid required for debridement using improved delivery devices and irrigation fluids. Because combat casualties have evacuation times that are longer than the typical civilian trauma patient, we are currently developing an animal model of combined bone and soft tissue damage and contamination. This model will include a delay after contamination and before irrigation to more closely mirror extended evacuation times. This model will serve as the test-bed for innovations that may reduce logistical loads such as use of potable water for irrigation, irrigation fluid additives (detergents or antimicrobials), pulsatile pressure delivery, and parallel flow delivery. In particular, we will investigate the ability of each of these technologies to meet or exceed the reduction in contamination provided by 10 liters of sterile saline.

Second, we are working to minimize the loss of salvageable tissue through the development of technologies to visualize tissue viability. After conducting an extensive survey of emerging technologies for noninvasively interrogating tissue, Optical Coherence Tomography was identified as the most promising. As there is already extensive industry and academic investment in the development of the core technology, the ISR entered into a partnership with the Beckman Laser Institute ([BLI], University of California, Irvine) to adapt this emerging technology to address the military medicine need. While the technical details of this technology are beyond the scope of this paper, devices in development at BLI have already shown promise in producing images of epidermis and endothelium with subcellular resolution. Research conducted at the ISR will attempt to determine if this imaging modality will be similarly successful in imaging other tissues, and whether these images will be useful as a diagnostic for tissue viability. Our technological goal is the development of a noninvasive, near real time, optical biopsy device which can be used to delineate margins of salvageable tissue. While this work is in the very early stages, a prototype device has been constructed and is currently under initial testing and validation.

Advanced Tourniquets. Though long out of favor, tourniquets for hemorrhage control when tactical or logistic restraints prevent immediate access to surgical intervention have been returned to use as self or buddy aid (for information regarding the recently fielded one-handed tourniquet, interested readers are referred to the article by Ryan et al, this issue). While tourniquets do provide a potentially lifesaving capability to the soldier, their use is not without risk. When discussing the use of tourniquets for far-forward use, the phrase "life over limb" is often used to describe the trade-off between lifesaving hemostasis and the potential for subsequent amputation of a limb after tourniquet use. Our goal is to minimize the negative impact of tourniquet application by conducting research on

several fronts, including investigating the pathophysiology of tourniquet injury, developing guidelines for nerve and muscle preserving tourniquet use guidelines, developing novel tissue salvage drug therapies, and designing a second generation tourniquet that is both hemostatic and soft tissue friendly. Readers interested in current research efforts on the effect of tourniquet injury and hemorrhage, and the development of protective use guidelines and therapies are referred to the article by Walters et al in this issue.

Conclusion

Though combat wounds cover virtually the entire spectrum of trauma, analysis of available statistics permits us to predict where soldier-focused research and development can have the greatest impact. Additionally, nascent trends in military doctrine such as the development of the Objective Force and an increased reliance on Special Operations units to shape the battlefield highlight the need for innovation in field medicine. The reduction of logistic loads to improve unit deployability and increased dispersion on the future battlefield will demand reduced reliance on medical evacuation and an increased effort to deliver initial medical care as far-forward as possible. By providing our soldiers with improved trauma care we seek to reduce the need for immediate medical evacuation from the battlefield while simultaneously mitigating the impact of extended evacuation. This, in turn, will reduce the adverse effects of injury, reduce workload and logistical requirements for theater medical assets, and increase the speed and number of soldiers returned to duty following wounding.

While current efforts are focused on those problems where we can achieve maximum impact in the near term, continued improvements in combat casualty care require us to advance our understanding of the pathophysiology of trauma. In addition to the research outlined above, investigators in the Bone and Soft Tissue program are involved in longer range research programs with the goal of improving wound healing after initial stabilization and repair of trauma. These efforts include characterizing factors which facilitate regrowth of bone following fracture, improving muscle regeneration to maximize recovery of strength, modulating nerve regeneration after injury (see article by Delgado et al this issue) and accelerating re-epithelialization of skin defects. We foresee that these lines of research will continue to advance the art and science of caring for combat casualties in order to minimize the impact of injury on our soldiers and the units that depend on them.

References

1. Maurer DJ, Merkow RL, Gustilo RB. Infection after intramedullary nailing of severe open tibial fractures initially treated with external fixation. *J Bone Joint Surg Am.* 1989;71:835-8.

2. McGraw JM, Lim EV. Treatment of open tibial-shaft fractures. External fixation and secondary intramedullary nailing. *J Bone Joint Surg Am.* 1988;70:900-11.

3. Campbell AA, Song L, Li XS, et al. Development, characterization, and anti-microbial efficacy of hydroxyapatite-chlorhexidine coatings produced by surface-induced mineralization. *J Biomed Mater Res.* 2000;53:400-7.

4. DeJong ES, DeBerardino TM, Brooks DE, et al. Antimicrobial efficacy of external fixator pins coated with a lipid stabilized hydroxyapatite/chlorhexidine complex to prevent pin tract infection in a goat model. *J Trauma.* 2001;50:1008-14.

5. Mabry RL, Holcomb JB, Baker AM, et al. United States Army Rangers in Somalia: an analysis of combat casualties on an urban battlefield. *J Trauma.* 2000;49:515-28; discussion 528-9.

6. Shouler PJ, Leicester RJ, Mellor S. Management of infections and complications during the Falkland Islands campaign. In: Gruber DF, ed. *The Pathophysiology of combined injury and trauma: management of infectious complications in mass casualty situations.* Orlando: Academic Press; 1987:43-51.

7. McBride JT. Report and Medical analyses of Personnel Injury from Operation "Just Cause." Letterman Army Institute of Research; 1991:86.

8. Uhorchak JM, Rodkey WG, Hunt MM, Hoxie SW. Casualty Data Assessment Team Operation Desert Storm. Letterman Army Institute of Research; 1992:121.

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An Antimicrobial Bone Graft Substitute

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Introduction

Background. The morbidity associated with open fractures and open fracture treatment is well established, especially on the modern battlefield. Open fractures that become infected nearly double the soldier's hospital stay. An antimicrobial, osteoconductive and osteoinductive bone graft substitute, if effective as an infection prophylaxis, would decrease the number of procedures required to treat contaminated open fractures by eliminating the need for antibiotic cement bead removal and for autograft harvest. Our hypothesis is that the combination of tobramycin-impregnated calcium sulfate pellets (Osteoset T®) and demineralized bone matrix (DBM) will be as effective as tobramycin-impregnated polymethylmethacrylate (PMMA) beads in preventing the establishment of infection in a contaminated deep tibial wound (open fracture model).

Methods. A unicortical 12 mm diameter defect was created in the proximal tibial metaphysis of 29 Spanish goats. After contaminating the wounds with 30 μL of 10^6 CFU/mL of a modified *S aureus (American Type Culture Collection [ATCC] 29213),* the animals were divided into four groups. The negative control group received no treatment, the positive control group received tobramycin-impregnated PMMA beads, the DBM group received 2.5 mL of DBM putty, and the treatment group received 15 Osteoset T® with 2.5 mL of DBM putty. After a 21-day evaluation period, radiographs, intraosseous tissue cultures, and histologic specimens were obtained.

Results. The treatment and the positive control groups had no microbiologic evidence of intramedullary infection; however, 6 of 7 goats in the negative control group and 7 of 8 in the DBM group had positive intramedullary cultures for streptomycin *S aureus*.

In the treatment group, 2 of 8 animals had superficial wound infections but no clinical signs of deep infection. None of the animals in the positive control group had clinical signs of infection. In the DBM group, 6 of 8 animals developed purulent culture positive wounds. Only 1 of 7 animals in the negative control group developed purulent discharge.

Discussion. The OsteoseT®, DBM combination was proven as effective as tobramycin-impregnated cement beads in preventing intramedullary *S aureus* infection in our contaminated open fracture model. Numerous studies support the use of local biodegradable antibiotic delivery systems. The combination of calcium sulfate pellets and DBM has been shown to be more effective in combination than either material alone. Likewise, the combination is as effective as autogenous bone graft at 6 weeks in a canine model. Our data demonstrate that the use of a locally delivered antibiotic impregnated in a bioabsorbable, osteoconductive, and osteoinductive combination can prevent establishment of *S aureus* infection in a contaminated open fracture. This approach could decrease the number of procedures and the morbidity to the soldier during the treatment of this common battlefield injury.

General

Open fractures are an extremely common injury on the battlefield. Frequently the result of high-energy impact, an open fracture involves skin and soft-tissue injuries that expose the fractured bone to the environment. Managing the associated bacterial contamination can often be an enormous surgical challenge, with considerable patient morbidity. Despite adequate treatment, open fractures have increased delayed union and nonunion rates, and complications like the development of chronic osteomyelitis can threaten the viability of the limb and possibly the life of the patient.[1] In addition, open fractures can often involve significant bone loss, resulting from the fracture comminution or from the surgeon's efforts to remove grossly contaminated and devitalized tissue. These bone defects typically require treatment with bone graft to augment fracture healing; however, the bone graft cannot be immediately placed in the wound due to the contamination and consequent high risk of infection.[2] The wound bed must first be thoroughly cleaned, which usually requires repeated surgical debridement and treatment with systemic and local antibiotics.

To help prevent establishment of infection in an open fracture, a well-established method of local delivery of antibiotics is through PMMA beads. Antibiotic-impregnated PMMA beads have been used for over 20 years in the treatment

of contaminated open fractures.[3,4] Local delivery of antibiotics has the advantage of high concentrations locally with low serum concentrations, thereby avoiding systemic toxicities. The PMMA beads, however, require a second surgery for removal, because they stop eluting antibiotic at 2-6 weeks and become a foreign body, and do nothing to assist healing.[4]

An ideal antibiotic delivery system would enhance healing, elute the entire antibiotic dose, and not require additional surgery for bead removal. There have been numerous animal studies showing biodegradable local antibiotic delivery systems as safe and effective treatments against osteomyelitis.[5-12] Recently, human trials by McKee and Gitelis have also shown that OsteosetT® are effective against chronic osteomyelitis.[13,14]

Calcium sulfate has been used since 1892 as bone defect filler and is known to be osteoconductive (acting as a scaffold for new bone growth).[15] Conversely, DBM is known to be osteoinductive, able to stimulate new bone cell growth.[15] Turner and Urban have shown that the combination of calcium sulfate pellets and DBM is more effective in stimulating bone growth in a canine model than either calcium sulfate or DBM alone.[16] In addition, they showed that the combination was just as effective as autogenous bone graft at 6 weeks following treatment.[16] Therefore, the combination of OsteosetT® and DBM could be used as a local antibiotic delivery system and be osteoinductive, osteoconductive, as well as antimicrobial.

The purpose of our study was to evaluate the ability of a locally delivered antimicrobial, osteoconductive, and osteoinductive combination in warding off infection in a contaminated, large animal, open fracture model. Our hypothesis was that the combination of OsteosetT® and DBM would be as effective as tobramycin-impregnated PMMA beads in preventing the establishment of infection in a contaminated deep tibial wound (open fracture model).

Materials and Methods

Animal General Procedures. All experiments and animal care procedures were approved by the Institutional Animal Care and Use Committee of the U.S. Army Institute of Surgical Research, Fort Sam Houston, TX. All procedures were conducted in an AAALAC approved animal facility according to the National Institutes of Health's "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources National Research Council.

Twenty-nine Spanish goats, with a weight range of 37 kg to 50 kg (42 kg ± 4 kg), were used in this study. All animals were housed in runs in a climate-controlled facility, and were fed commercial food and water ad libitum. In addition to being tested for tuberculosis, brucellosis, and Q fever, all animals were observed for 10 to 14 days prior to the study to allow for environmental changes and to exclude the possibility of preexisting disease. Animals were examined by a veterinarian prior to commencement of the protocol.

Surgical Technique. Prior to surgery, animals were fasted for 48 hours and water was withheld for 12 hours. After the animals were sedated with ketamine hydrochloride (2.2-7.0 mg/kg IM) and midazolam (0.125-0.250 mg/kg IM), an endotracheal tube was inserted and general anesthesia was induced with a mixture of isoflurane and oxygen. Oxygen saturation, heart rate, ventilatory rate, end tidal CO_2, and agent concentration were monitored. In addition, all animals received epidural analgesia using morphine sulfate prior to surgery for post-operative pain control.

After adequate regional and general anesthesia was achieved, the right lower extremity was prepped with Hibiclens® and draped in a sterile fashion. To avoid confounding variables, no preoperative intravenous antibiotics were given. A 2.5 cm longitudinal incision was made over the medial proximal metaphyseal region of the tibia centered at a point 2 cm medial and 2 cm distal to the tibial tubercle. Electrocautery was used to maintain hemostasis. After elevating the periosteum with a periosteal elevator, a unicortical, 12 mm circular defect was produced with a coring reamer. Thrombin-soaked gel foam was used to assist in medullary hemostasis. Next, a sterile pipette containing an aliquot of bacteria (30 μL of solution with a mean of 3.14×10^6 CFU/mL of *S aureus*) was used to inoculate the bony defect. The bacterial strain used was ATCC 29213, and was further modified by our institution to be resistant to streptomycin. This amount of bacteria has been shown in our previous studies to be sufficient to cause infection in greater than 70% of the animals without producing overwhelming sepsis.[17] After inoculation, the defects underwent one of four designated treatments.

Before starting the study, 48 animals were randomized and balanced into four groups of 12. Midway though data collection a pre-planned interim statistical analysis halted the study resulting in 2 equal groups of 8 animals, one group of 7 and 1 group of 6 animals. The first group, negative control, (n = 7) received no treatment. The second group, positive control, (n = 6) was treated with tobramycin-impregnated PMMA beads. These beads were made by combining one packet (40 gm) of PMMA cement (Palacos®, Biomet, Warsaw, IN) with 2.4 g of tobramycin powder. Fifteen to 19 of these beads were placed into the bone defect of each animal resulting in a dose of 160.6 ± 10.3 mg of tobramycin sulfate. In the third group (n = 8) 2.5 mL of DBM (Allomatrix® injectable putty, Wright Medical, Arlington, TN) was placed in the metaphyseal defect. The

treatment group (n = 8) received 15 pellets of 10% tobramycin-impregnated calcium sulfate (Osteoset T®, Wright Medical, Arlington, TN) with 2.5 mL of DBM. 15 pellets of Osteoset T® has a total of 160 mg of tobramycin sulfate.

After receiving the designated procedure, the skin was re-approximated with surgical staples and a dry, sterile dressing was applied. After emerging from anesthesia, the endotracheal tube was removed and the animal was closely monitored and kept warm until fully recovered.

Wound Evaluation. The animals were followed daily for 21 days for clinical signs of infection. The operatively placed sterile dressing remained intact until post-operative day 4, at which time the dressing and the staples were removed. If, during the daily wound examination and scoring (see below), the wound displayed excessive bleeding or drainage, a light compressive gauze dressing and Vetwrap® was applied and removed the following day. Abscess formation was followed clinically by measuring leg circumference. Abscesses that increased in size over 3 days were relieved with aspiration or incision and drainage. Abscesses requiring incision and drainage or aspiration were cultured and sent for qualitative analysis. Likewise, at the first sign of purulent drainage, the wound was cultured and analyzed for bacterial type. All *S aureus* isolates were tested for streptomycin resistance to determine if the cultured strain was identical to the original, inoculated bacteria. Finally, on post-operative day 21, all of the animals were euthanized and necropsy was performed as described below.

During the 3-week observation period, if any animal demonstrated discomfort or distress, a fentanyl citrate patch (Duragesic®, 100 μg/h) was placed on the skin of the neck area and secured with Vetwrap® under veterinarian supervision. These patches were effective for 72 to 96 hours and were reapplied as needed.

Wound Grading System. Clinical signs of wound infection included erythema, inflammation, and purulent drainage. After the dressing was removed on post-operative day 4, each wound was scored daily by 3 independent examiners. The goat's treatment group was masked to the graders for the duration of the study. The clinical grading system used was established in previous studies conducted in this laboratory.[17] The condition of the wound was graded by the following criteria: 0 – demonstrating no signs of contamination; 1 – showing inflammation, swelling, or serous drainage without frank purulence; and 2 – demonstrating frank purulence at the wound site, or obtaining purulent discharge upon aspiration or incision and drainage. A score for each wound was calculated by adding the score from each of the three observers each day for 21 days. The clinical determination of infection was defined by a score of 5 on two consecutive days for a given wound. This required a wound to exhibit 2 consecutive days of purulent drainage, as identified by 2 of 3 examiners, to be considered infected.

Necropsy/Microbiologic Analysis. At 3 weeks post-operatively, the animals were euthanized and the hind limbs were disarticulated at the hip. Radiographs of the treated hind limb were performed. Next, soft-tissue was removed from the tibia using sterile technique, and the bony defect was transected at its mid portion with a gigli saw. Ex vivo, the bone was protected from contamination by cleaning the surface of the outer cortex with alcohol-soaked gauze prior to cutting through the cortical defect with a sterile saw. After obtaining two more culture swabs from the proximal and distal intramedullary canals, a No. 5 surgical curette (0.5 g tissue) was used to harvest marrow and trabecular tissue from the canal.

These tissue and swab samples were sent for standard quantitative and qualitative microbiological analysis. Each aliquot was then removed and placed into 10 mL of phosphate buffered saline with 0.01% trypsin. The material was vortexed for 30 seconds, then sonicated for 3 minutes to remove the bacteria from the material. Quantitative bacterial counts were done by the spread plate method on trypticase soy agar. Isolation and identification of the bacterial species was performed on MacConkey agar plates and trypticase soy agar plates with 5% sheep blood over 48 hours. Isolates were identified by routine microbiological procedures. Each *S aureus* isolate was tested for streptomycin resistance to determine whether it was the same streptomycin resistant strain as the initial inoculation.

Finally, 2 cm portions of the tibia just proximal and distal to the bony defect was removed with a sagittal saw and analyzed histologically by a pathologist.

Outcome Measures. The primary outcome measure for deep wound infection was the recovery of the streptomycin resistant *S aureus* strain ATCC No. 29213 from intramedullary cultures at 21 days. The threshold for infection was set at 10^4 CFU/g of marrow. Wounds with bacteria present but with less than 10^3 CFU/g marrow of bacteria at final tissue culture, the presence or absence of deep wound infection was confirmed by our clinical score for that animal.

Results

In the negative control group, we were able to confirm intramedullary infection with streptomycin resistant *S aureus* in 6 of the 7 animals with a mean of 2.2×10^8 CFU/g (table). During the clinical evaluation period of the control group, one animal developed purulent drainage and received a score of 5 in our wound grading scale and one animal, with negative cultures,

Group	Clinical exam	Bone culture	Wound culture*
Neg Control	Purulent discharge	*S aureus*	*S aureus* post-op day 4
Neg Control	Edema	*S aureus*	
Neg Control	Healing wound	No growth	
Neg Control	Edema	*S aureus*	
Neg Control	Increasing edema	*S aureus*	
Neg Control	Fluctuance	*S aureus*	
Neg Control	Healing wound	*S aureus*	
Pos Control	Healing wound	No growth	
Pos Control	Healing wound	No growth	
Pos Control	Healing wound	No growth	
Pos Control	Healing wound	No growth	
Pos Control	Healing wound	No growth	
Pos Control	Healing wound	No growth	
DBM	Purulent discharge	*S aureus*	*S aureus* post-op day 4
DBM	Edema	*S aureus*	*S aureus* post-op day 4
DBM	Purulent discharge	*S aureus*	*S aureus* post-op day 6
DBM	Purulent discharge	*S aureus*	*S aureus* post-op day 7
DBM	Purulent discharge	*S aureus*	*S aureus* post-op day 7
DBM	Healing wound	*S aureus*	
DBM	Purulent discharge	*S viridans*	*S aureus* post-op day 5
DBM	Purulent discharge	*S aureus*	*S aureus* post-op day 5
Treatment	Early serous discharge	No growth	
Treatment	Early serous discharge	No growth	
Treatment	Healing wound	No growth	
Treatment	Early serous discharge	No growth	
Treatment	Healing wound	No growth	
Treatment	Healing wound	No growth	
Treatment	Early serous discharge	No growth	Non-hemolytic Strep post-op day 21
Treatment	Healing wound	No growth	

*Table. Clinical and Culture Results. *Cultures Done During Clinical Evaluation DBM*

was graded as normal with a score of 0 (table). All the animals in this group had periosteal reaction on final radiographs (Figure 1). Gross pathology demonstrated necrosis and abscess formation in 5 of 7 animals (Figure 2).

In the DBM group, we were able to confirm intramedullary infection with streptomycin resistant *S aureus* in 7 of 8 animals with a mean of 1.3×10^8 CFU/g (table). Early in the 21-day observation period, one animal had streptomycin resistant *S aureus* culture positive purulent discharge, but final culture was found to have *Streptococcus viridans* in the intramedullary tissue. The clinical signs of infection were considerably more evident in this group compared with the negative control in which 6 of 8 animals developed purulent discharge and had wound scores of 5 (table). All animals had periosteal elevation on radiographic exam (Figure 3). Gross pathology demonstrated abscesses, necrosis, and draining sinuses in 7 of 8 animals (Figure 4).

We were unable to culture streptomycin resistant *S aureus* from the intramedullary tissues of the 6 animals in the positive control group (tobramycin-impregnated PMMA beads). During the 21 day clinical evaluation period, 6 of 6 animals healed without any signs of infection and received a 0 wound score (table). Likewise, none of the animals had periosteal reaction on final radiographs (Figure 5). Gross pathologic exam showed no signs of infection (Figure 6).

We were unable to culture streptomycin resistant *S aureus* from the intramedullary tissues of the 8 animals in the treatment control group (OsteosetT® with DBM). During the clinical evaluation period, 4 of the 8 animals had 2 to 6 days of early,

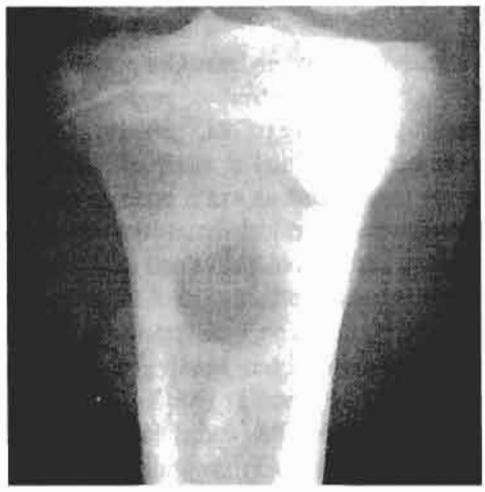

Fig 1. Representative radiograph of negative control group, note cortical defect and periosteal elevation.

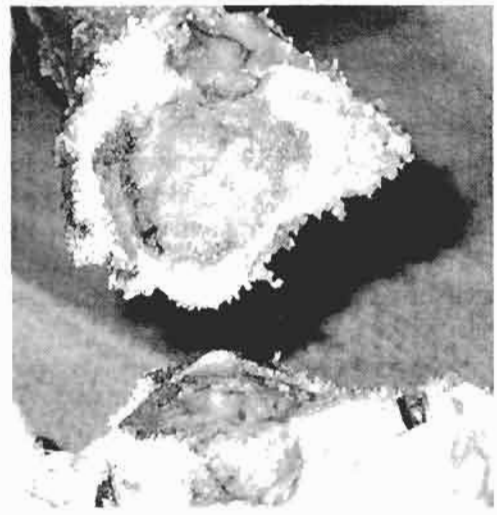

Fig 2. Representative gross pathology of negative control group, note intramedullary purulence.

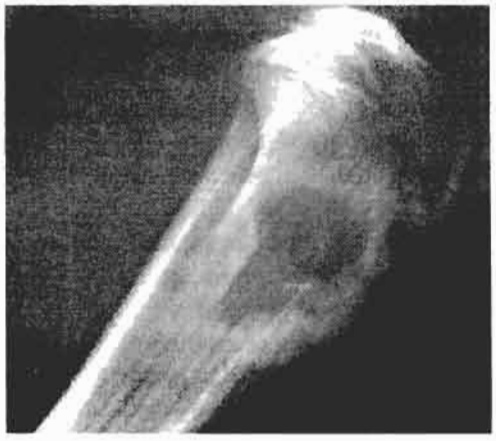

Fig 3. Representative radiograph of DBM group, note cortical defect and periosteal elevation.

Fig 4. Representative gross pathology of DBM control group, note intramedullary purulence.

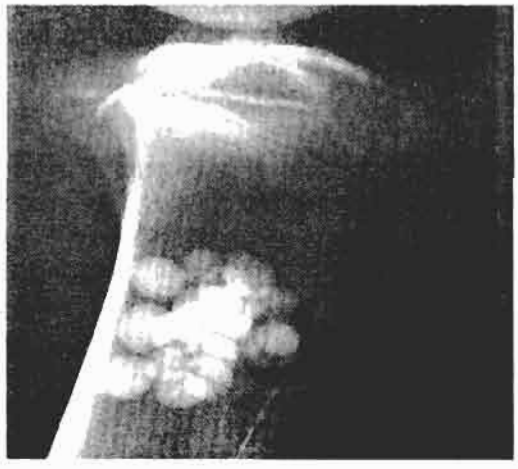

Fig 5. Representative radiograph of PMMA group, note cortical defect and cement beads.

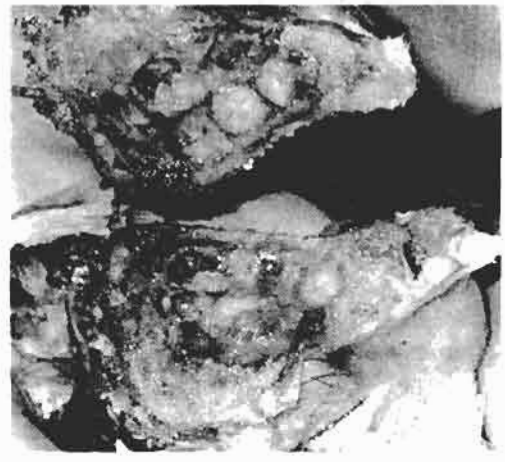

Fig 6. Representative gross pathology of PMMA control group, note cement beads.

culture negative, serous discharge. Two animals in the treatment group had superficial wound infections; one was infected with nonhemolytic *Streptococcus*, and the other animal's infection resolved after removal of a small eschar early in the evaluation period (table). Radiographic exam revealed periosteal elevation in 6 of 8 animals, however, the degree of reaction was considerably less than the negative control and the DBM groups (Figure 7). Gross pathologic examination demonstrated incorporation of DBM and calcium sulfate pellets without evidence of infection (Figure 8).

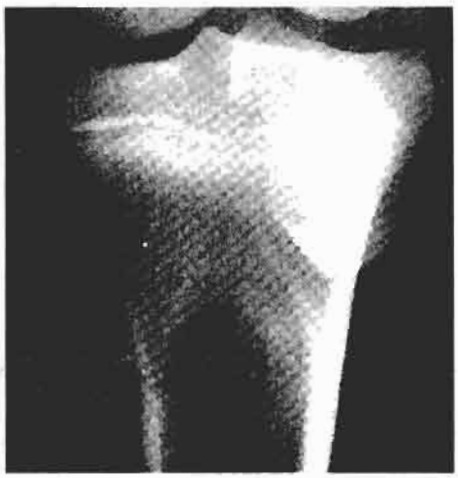

Fig 7. Representative radiograph of treatment group, note calcium sulfate pellet incorporation and mild periosteal elevation.

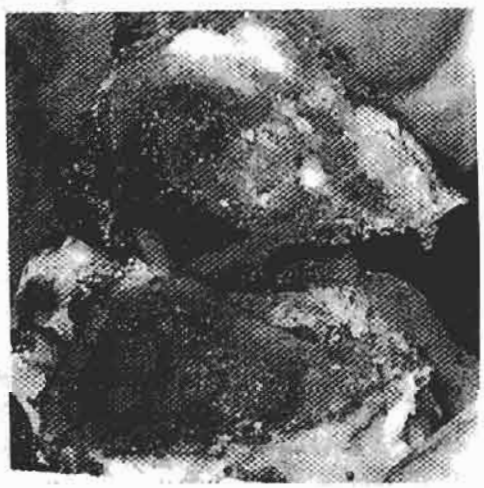

Fig 8. Representative gross pathology of treatment control group, note pellet incorporation.

Discussion

Previous animal studies and human clinical trials have shown Osteoset T® to be effective against osteomyelitis.[7,13,14,18] Since biodegradable antibiotic drug delivery systems are effective against osteomyelitis, it stands to reason that they might be effective as a prophylaxis. Our previous work showed OsteosetT® alone is an effective prophylaxis in a caprine contaminated open fracture model.[19] However, calcium sulfate is only osteoconductive and it takes 24 weeks for calcium sulfate pellets to stimulate as much bone as autogenous bone graft.[16] The combination of calcium sulfate pellets and DBM is osteoconductive and osteoinductive and can stimulate as much bone growth as autograft in just 6 weeks.[16] By adding tobramycin, the combination is antimicrobial as well, but this combination had never been challenged in a large animal contaminated fracture model. Our results show that the combination of OsteosetT® and DBM is as effective as tobramycin PMMA beads in preventing establishment of *S aureus* infection in a contaminated open fracture model, thereby confirming our hypothesis.

We observed 2 to 6 days of serous drainage in the treatment group. Similar drainage was observed in a clinical trial, in which the investigators noted that the drainage halted upon radiographic resorption of the calcium sulfate pellets.[13] We feel this drainage could explain the two superficial wound infections in our treatment group, as our patients were not as invested in wound care as human patients.

By eliminating the need for surgical bead removal and by reducing the number of autografts taken, such a treatment will decrease the morbidity of open fracture treatment. Autologous bone graft harvest, typically taken from the patient's iliac crest, may result in significant morbidity including: increased blood loss, prolonged operative time, persistent post-operative pain, chronic wound drainage, and difficulty ambulating.[20,21] Developing a safe and effective alternative to autologous bone grafting would help alleviate many of these problems. In addition, this approach could reduce morbidity through decreasing the number of infected open fractures. Infected open fractures double the hospital stay, especially in combat wounds.[22]

Our results indicate a bioabsorbable osteoinductive, osteoconductive antibiotic-impregnated combination can be used acutely in a contaminated open fracture. If further studies demonstrate a decrease in nonunion rates, our approach could be an effective substitute for autograft. This approach could likewise decrease osteomyelitis rates. Further study will therefore focus on comparing our approach with other standards of care (for example, PMMA beads with intravenous antibiotics) to determine whether such a combination will produce a decrease in chronic osteomyelitis and nonunion rates.

References

1. Maricevic A, Erceg M. War injuries to the extremities. *Mil Med.* 1997;162:808-11.

2. Gustilo RB, Mendoza RM, Williams DN. Problems in the management of type III (severe) open fractures: a new classification of type III open fractures. *J Trauma*. 1984;24:742-6.

3. Buchholz HW, Elson RA, Heinert K. Antibiotic-loaded acrylic cement: current concepts. *Clin Orthop*. 1984:96-108.

4. Ostermann PA, Seligson D, Henry SL. Local antibiotic therapy for severe open fractures. A review of 1085 consecutive cases. *J Bone Joint Surg Br*. 1995;77:93-7.

5. Aimin C, Chunlin H, Juliang B, Tinyin Z, Zhichao D. Antibiotic loaded chitosan bar. An in vitro, in vivo study of a possible treatment for osteomyelitis. *Clin Orthop*. 1999:239-47.

6. Calhoun JH, Mader JT. Treatment of osteomyelitis with a biodegradable antibiotic implant. *Clin Orthop*. 1997:206-14.

7. Dahners LE, Funderburk CH. Gentamicin-loaded plaster of Paris as a treatment of experimental osteomyelitis in rabbits. *Clin Orthop*. 1987:278-82.

8. Humphrey JS, Mehta S, Seaber AV, Vail TP. Pharmacokinetics of a degradable drug delivery system in bone. *Clin Orthop*. 1998:218-24.

9. Mader JT, Stevens CM, Stevens JH, Ruble R, Lathrop JT, Calhoun JH. Treatment of experimental osteomyelitis with a fibrin sealant antibiotic implant. *Clin Orthop*. 2002.58-72.

10. Mehta S, Humphrey JS, Schenkman DI, Seaber AV, Vail TP. Gentamicin distribution from a collagen carrier. *J Orthop Res*. 1996;14:749-54.

11. Rogers-Foy JM, Powers DL, Brosnan DA, Barefoot SF, Friedman RJ, LaBerge M. Hydroxyapatite composites designed for antibiotic drug delivery and bone reconstruction: a caprine model. *J Invest Surg*. 1999;12:263-75.

12. Solberg BD, Gutow AP, Baumgaertner MR. Efficacy of gentamycin-impregnated resorbable hydroxyapatite cement in treating osteomyelitis in a rat model. *J Orthop Trauma*. 1999;13:102-6.

13. McKee MD, Wild LM, Schemitsch EH, Waddell JP. The use of an antibiotic-impregnated, osteoconductive, bioabsorbable bone substitute in the treatment of infected long bone defects: early results of a prospective trial. *J Orthop Trauma*. 2002;16:622-7.

14. Gitelis S, Brebach GT. The treatment of chronic osteomyelitis with a biodegradable antibiotic- impregnated implant. *J Orthop Surg (Hong Kong)*. 2002;10:53-60.

15. Greenwald AS, Boden SD, Goldberg VM, Khan Y, Laurencin CT, Rosier RN. Bone-graft substitutes: facts, fictions, and applications. *J Bone Joint Surg Am*. 2001;83-A:98-103.

16. Turner TM, Urban RM, Gitelis S, Kuo KN, Andersson GB. Radiographic and histologic assessment of calcium sulfate in experimental animal models and clinical use as a resorbable bone-graft substitute, a bone-graft expander, and a method for local antibiotic delivery. One institution's experience. *J Bone Joint Surg Am*. 2001;83-A:8-18.

17. DeJong ES, DeBerardino TM, Brooks DE, et al. Antimicrobial efficacy of external fixator pins coated with a lipid stabilized hydroxyapatite/chlorhexidine complex to prevent pin tract infection in a goat model. *J Trauma*. 2001;50:1008-14.

18. Bowyer GW, Cumberland N. Antibiotic release from impregnated pellets and beads. *J Trauma*. 1994;36:331-5.

19. Thomas DB, Lonergan KT, Brooks DE, DeJong S, McManus AT, Deberardino TM. *Treatment of a Contaminated Tibial Defect with an Antimicrobial Bone Graft. Meeting of the Society of Military Orthopedic Surgeons*. San Diego CA; 2002.

20. Arrington ED, Smith WJ, Chambers HG, Bucknell AL, Davino NA. Complications of iliac crest bone graft harvesting. *Clin Orthop*. 1996:300-9.

21. Younger EM, Chapman MW. Morbidity at bone graft donor sites. *J Orthop Trauma*. 1989;3:192-5.

22. Mabry RL, Holcomb JB, Baker AM, et al. United States Army Rangers in Somalia: an analysis of combat casualties on an urban battlefield. *J Trauma*. 2000;49:515-28. Ddiscussion 528-9.

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Nerve Regeneration and Wound Healing: Implication in Combat Casualty Care

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Albert T. McManus, PhD††

Wound care management is a critical component to the future success of far-forward, and en-route care for soldiers wounded on the battlefield. Improved methods will enable the combat medic to focus more time on the most seriously wounded while enabling other casualties to defend themselves and or participate in self-care. The Institute's Combat Casualty Care Research Program emphasizes delivery of immediate, far-forward, and en-route care for soldiers wounded on the battlefield. This protocol applies directly to two identified combat casualty care research areas: neuroprotection and soft tissue injury. For decades, research to improve wound healing following traumatic skin injury has focused primarily on the repair of dermis and endothelium, with little attention to the repair of the nerves that innervate the wounds. Nerves contain and release a variety of biologically active compounds. These compounds can be of potential functional significance during injury repair. Injury induces a sequence of neuropeptide responses in wounds. The ability to affect proliferation of various types of target cells and to improve healing of *experimentally malperfused tissues suggests a regulatory role of neuropeptides in tissue repair. Substance P (SP) is one of the neuropeptides that shows significant promise. Also, neurogenic mechanisms that govern local blood perfusion, oxygenation to the wound site, and axonal regeneration may play an important role in determining the rate of healing. We describe a laser induced skin wound model that will be used in future studies designed to screen therapeutic agents such as the neuropeptide SP and techniques that may improve the rate of axogenesis in healing tissue.*

Introduction

As we begin to understand the mechanisms and the conditions required for nerve tissue regeneration, we move towards the ultimate goal of treating nerve tissue injuries. This includes the simple peripheral dysfunction of hypo or hyperexcitable nerve endings and complex brain and spinal cord injuries. Successful repair of injured tissues requires diverse interactions between cells, biochemical mediators, and the cellular microenvironment.[1-3] Much has been learned about the individual events that are involved in this process, but their integration is clearly far more complex than has been imagined, and the important role of neurogenic stimuli to repair the nerves that innervate the wound has only recently been recognized.

Neurogenic stimuli profoundly affect cellular events that are involved in inflammation, proliferation, and matrix plasticity, as well as cytokine and growth factor synthesis. Neuropeptides mediate many of the actions important in tissue-nervous system communication. Immune cells regulated by neuropeptides include lymphocyte subsets, macrophages, and mast cells. In addition, neuropeptides may affect the proliferative and synthetic activity of epithelial, vascular, and connective tissue cells. Furthermore, recent investigations have revealed a strong interaction between the nervous and immune systems.[4] The peripheral nervous system (PNS), acting through neuropeptides, not only relays sensory information to the central nervous system (CNS) but also plays an effector role in the inflammatory, proliferative, and reparative processes after injury. These effects range from growth factor and cytokine responses to control of local blood flow.

In contrast to the classic low molecular weight neurotransmitters, neuropeptides are exclusively produced in the cell soma without local synthesis in nerve endings.[5] In most instances, several different neuropeptides are encoded by a single continuous messenger RNA, which is translated into one large protein precursor (polyprotein). Like other secretory proteins, neuropeptides or their precursors are processed in the endoplasmic reticulum and then move to the Golgi apparatus to be processed further. Neuropeptides leave the Golgi apparatus within secretory granules and are transferred to terminals by fast axonal transport.[6]

In the PNS, neuropeptides occur in the perivascular terminals of noradrenergic (sympathetic) and noncholinergic nerve fibers, as well as in the free nerve endings of primary afferent neurons.[7,8] Numerous neuropeptides are localized in nociceptive afferent nerve fibers, including thinly myelinated A-delta pain fibers and unmyelinated C fibers.[9] Antidromic stimulation of these fibers induces the release of the stored neuropeptides, resulting in vasodilation, increased vascular permeability, and edema (neurogenic inflammation).[8,10] These effects are not restricted to the point of the initial stimulus but also can be observed in the surrounding area, indicating that the nerve impulses travel not only centrally, but at the collateral branches also pass antidromically to unstimulated nerve endings to cause release of neuropeptides (axon reflex).[8]

It has been shown that nonadrenergic noncholinergic (NANC) neurons use neuropeptides such as SP, neurokinin A, and calcitonin gene-related peptide as transmitters in many vascular beds to mediate vasodilatation.[11] The perivascular NANC neurons are sensory neurons capable of monitoring the chemical and physical environment and are these primary afferent neurons that convey this information to the CNS. We will attempt to determine this role.

Cutaneous vasodilatation results from antidromic conduction of nerve impulses in afferent nerve fibers.[12] This antidromic vasodilatation is associated with an increase in vascular permeability leading to extravasation of plasma proteins and formation of edema.[13] Hyperemia and increased vascular permeability are key traits of inflammation. The SP does not evoke vasodilatation directly but through the release of histamine from dermal mast cells in human skin.[14] It has been proposed that activated mast cells release cytokines and stimulate fibroblasts to secrete nerve growth factor (NGF). This NGF will, in turn, stimulate the sensory nerve soma to produce more SP.[15] This system is a proposed positive feedback mechanism for axogenesis (Figure 1).

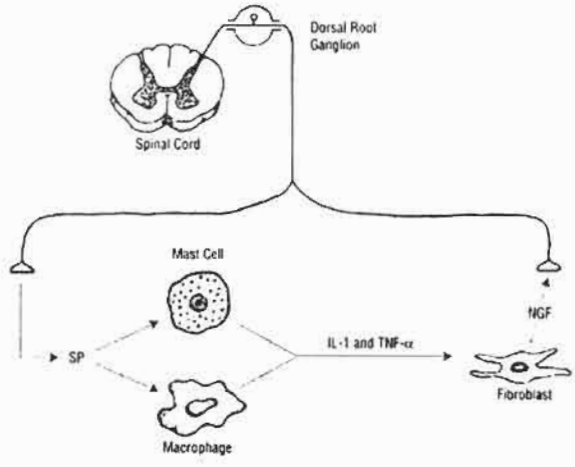

Fig 1. Substance P activates mast cells, which release cytokines (IL-1 and TNF-a) stimulating fibroblasts to secrete NGF which, in turn, further stimulates production of SP.

Evidence indicates that intradermal injection of SP in human skin produces flare and itching.[16] In clinical practice, temporary itching is an indication of the re-epithelialization period in wound healing. The flare component of the inflammation response is attributed to cellular infiltration of the injured site, especially neutrophil accumulation.[9] There are reports that SP induces chemotaxis of neutrophils in rats.[16] The SP also induces cytokine release by macrophages and neutrophils.[17]

The SP-containing axons are primarily found in the connective tissue surrounding the vessels and at the border between adventitia and media (muscle layer) as well as in the dermis and epidermis of the rat.[13,18,19] The SP is also present in endothelial cells.[20] There is strong evidence showing that injured nerve endings release SP in support of neurogenic mediation of inflammation and vasodilatation in early wound repair. This is supported by the absence of SP fibers from epidermis and extracellular matrix in early burn wounds and the repopulating of the wound bed with SP fibers which followed neovascularization originating in the deep reticular dermis and wound edge.[21]

Healing of injured tissues requires adequate organizational and functional reactions of the primary afferent sensory nerves and the microcirculatory system. Insufficient or improper coordination of these processes may predispose a wound to trophic disorders of the skin. Support for this conjecture comes from clinical observations that sensory neuropathies may be associated with persistent skin ulcers and connective tissue diseases.[22,23] The SP is in fact able to induce neovascularization of the vascular rabbit cornea and is also known to cause neurite outgrowth in embryonic chick dorsal root ganglia.[24,25] These actions of SP are likely to play a role in the angiogenesis of healing tissue. The SP and CGRP exert potent proliferative stimuli on cultured fibroblasts.[26,27] These data suggest that neuropeptides released from peripheral nerve endings in association with tissue injury may not only affect vasodilation and the inflammatory response, but may also stimulate proliferation of epithelial, vascular, and connective tissue cells. Lastly, neurogenic mechanisms that govern local blood perfusion and oxygenation may profoundly affect the rate of healing. The affected processes include angiogenesis, collagen deposition, bacterial killing, and epithelialization. Dysfunction of sensory afferent neurons is likely to have a bearing on the pathophysiology of the skin.

Study Objectives and Hypothesis

The objectives of this study were (1) to develop and characterize a laser-ablation skin-wound model for wound axonal growth, (2) to perform an initial test (one dose) of SP as a potential agent to modify wound axonal growth, and (3) to obtain variance information to be used to determine animal requirements in further protocols investigating other agents that may modify axonal growth. Our second objective was prompted by the hypothesis that SP alters wound axonal growth.

Materials and Methods

For this study, Sprague-Dawley rats (350 - 400 g) were used. All animals were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care

International accredited facility. The Animal Care and Use Committee of the U.S. Army Institute of Surgical Research, Fort Sam Houston, TX, approved the study. All animals received care in strict compliance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996).

Before injury, all animals received the preanesthetic analgesic ketoprophen (200 mg/kg, IM). A surgical plane of anesthesia was induced using inhalation anesthetic (Isofluorane gas) to effect. Post-operatively additional analgesics were administered as needed under the direction of the veterinary staff.

Rats have patches of telogen-phase (thinner and less vascular) skin, in juxtaposition to patches of anagen-phase (thicker and more vascular) skin in patterns that vary between rats. For the laser ablation to proceed to a consistent depth with respect to important landmarks after a given amount of energy is applied, the intended sites of lesions must be brought to the same hair-cycle phase and skin thickness. This was achieved according to the methods of Zawacki and Jones.[28] Thirteen days before the intended laser procedure, we clipped the hair on the back of the rat in (and just wide of) the intended laser wound sites and applied barium sulphide depilatory cream in order to stimulate telogen sites into anagen phase and bring the skin to a uniform thickness by the time the laser wound is applied. On the day of surgery, the laser injury area was depilated once more before inducing the wound.

For creating the injury in this study a 150XJ CO_2 laser equipped with a computer controlled x, y galvanized scanner and a 260 mm focal length hand piece (Sharplan, Allendale, NJ) was used (Figure 2). The laser was set at 120 W with a beam delivery pattern of 100 mm^2 and pulse duration of 0.45 seconds. This generates 54 J/cm^2 of energy per pulse delivered. The time interval between the delivered pulses was 2 seconds. All safety precautions were observed such as safety eyewear, norminal zone precautions, fire hazards, and the use of a smoke (plume) evacuator.

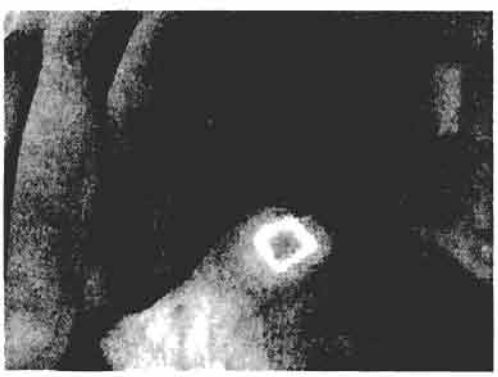

Fig 2. XJ150 CO_2 laser generating a 100 mm^2 deep dermal injury.

For this study, SP 10^{-6}M (200 μL) (718.9 ng/kg) was pipetted topically to the wound after injury. Skin samples, at selected time points, of each rat were collected and placed in formalin for histological assessment. The staining of nerve axons was as follows: tissue sections were fixed with formalin and embedded in paraffin. The antibody, rabbit anti-neurofilament 200 (NF-200), was used to detect axons. After incubation with the primary antibody, a fluorescent-labeled secondary antibody was applied to the tissue sections. The detailed immunohistochemistry procedure is described by McCarthy et al.[29] Acquisition of images of stained samples was done using a fluorescent confocal microscope.

Quantitative assessment of axonal growth were analyzed two ways: (1) fluorescent axons were counted in 10 different well-defined specific regions of the tissue sample. The number of axons in each region was summed; (2) total mean fluorescent intensity of each sample was quantitated and used as an index of axonal measurement. Both methods were done using MetaMorph® (Nikon, Lewisville, TX) an integrated imaging system.

Findings

During the method development phase of this study it was determined that the laser energy needed to generate the required injury was between 378 and 486 J/cm^2. Phase 1 was then initiated in order to optimize the energy required to generate a reproducible deep dermal injury that will remove 100% of the epidermis and about 50% of the dermis. The histological data showed that the required energy is 378 J/cm^2 delivered in 7 pulses of 54 J/cm^2 per pulse. The data also showed that the position of the injury makes a difference in wound depth. All wounds were done in the middle of the animals back not on the rostral or caudal areas. The laser settings for the remainder of the study have been determined to be: power 120 W, focal distance 260 mm, beam delivery pattern 100 mm^2, pulse duration 0.45s, energy density per pulse 54 J/cm^2, number of pulses 7, between pulse duration 2.0s. At these laser settings, we see that the injuries show a 50% dermal collagen depth affected by the laser injury and that deep epithelial structures remain unaffected.

Once we had established a reproducible injury, phase 2 was initiated in order to characterize (1) the wound (grossly as well as histologically), (2) normal axonal outgrowth patterns into the wound, and (3) the time course of the wound (0, 7, 14, and 21 days post injury). Grossly, the wound showed no bleeding, crust formation by days 8-10, no evident discharge, no

signs of infection, no evidence of tissue separation, and a mild erythema on days 3-8. This shows that this model has no confounding mediated by infection or inflammation. Additionally, we performed daily pain assessment on all animals. The animals showed no signs of pain or distress as they exhibited normal activity (grooming), normal posture, and normal appetite and weight gain during the period following the injury. The histological data show consistent control skin depth of 2.05 + 0.04 mm and after laser ablation a viable skin depth of 0.95 + 0.07 mm. The wound showed signs of healing by day 7 post injury as well as slight contraction. Scab formation was evident by day 14, with re-epithielithation of the wound underneath the scab. By day 21 post injury, the wound appeared healed at the gross level, but still showed signs of healing at the histology level compared to uninjured control. The axonal outgrowth determination showed a clear decrease in stained axons at the injury site post laser ablation. The number of neurites increased with time after injury (Figure 3). This preliminary data analysis indicated that day 4 post injury is the optimal time point to examine neurite outgrowth. Phase 3 was completed and the data acquired from this phase helped to determine variance for statistical purposes. We also assessed the effect of one initial concentration of SP in this model, which demonstrated improvements in exogenesis and wound healing.

Conclusion

We believe that we have a valid, reproducible model of nerve skin injury as well as a potential therapy for the acceleration of exogenesis rate. The studies performed to date characterize the injury and establish specific parameters for the monitoring of healing in this injury model. Likewise, we are

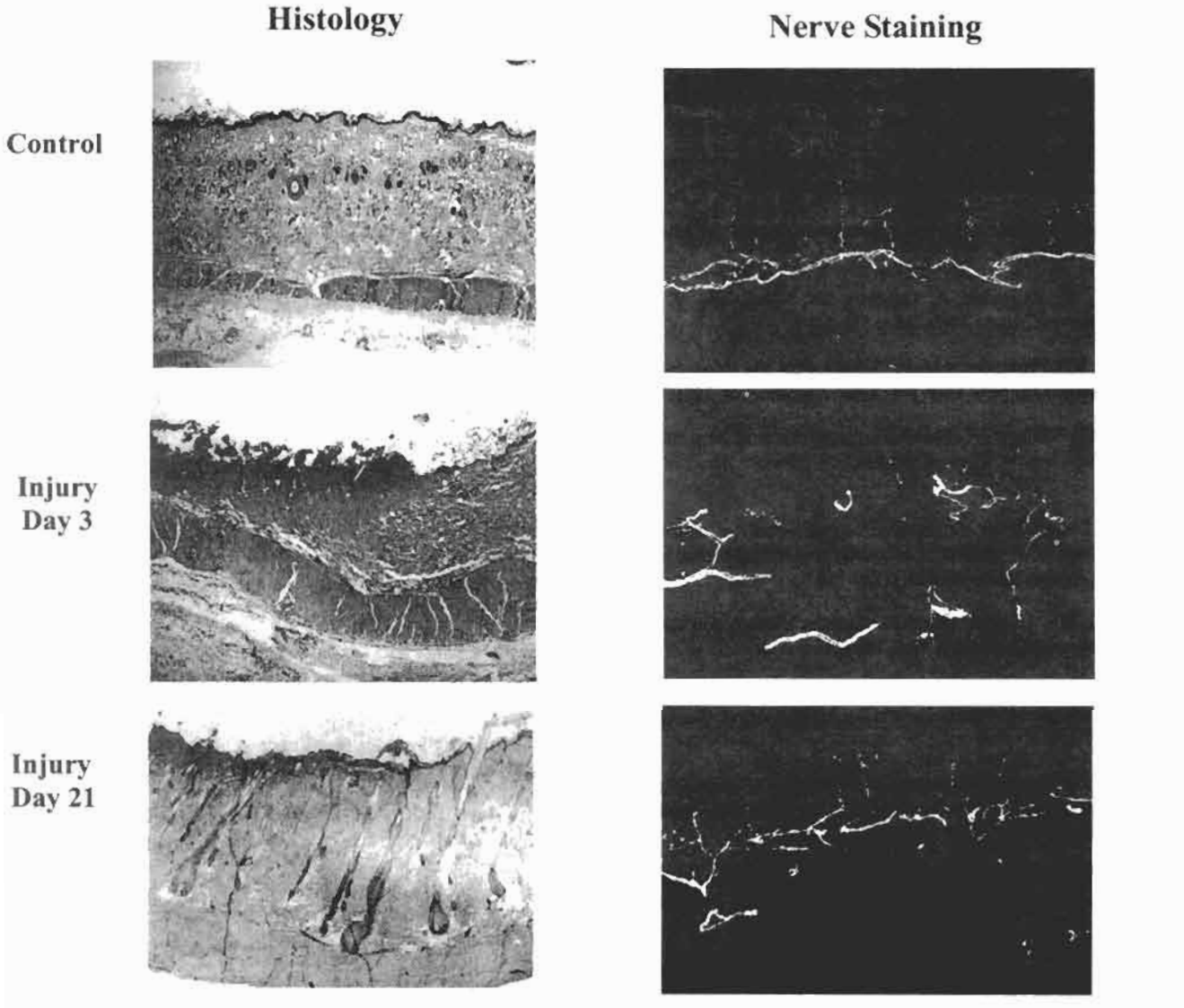

Fig 3. Histological determination of laser injury in time. H&E staining comparison to neurite outgrowth staining.

elucidating the mechanisms involved in repair of nerve injury. In the future, we plan to evaluate the effect of SP on axogenesis and wound healing in a dose-dependent manner. We will also determine the specificity of SP by pretreating a group of animals with an SP antagonist prior to SP treatment. This will be done in order to validate the direct effects of SP. We are also going to study the pattern of gene regulation as a function of SP administration in order to determine if any gene or group of genes are responsible for the rate of axogenesis. Once we learn the mechanisms that control the rate of nerve regeneration and wound healing we will be able to modulate this rate and enhance the current time frame it takes for these injuries to heal. The faster the rate of healing , the quicker a casualty is relieved of pain and returned to duty.

References

1. AB. Immune aspects of wound repair. *Clin Plast Surg.* 1990;17: p 433-442.

2. Cioffi WG, BD, Pruitt BA. Leukocyte responses to injury. *Arch Surg.* 1993;128:p 1260-1267.

3. Guirao X, LS. Biologic control of injury and inflammation: much more than too little or too late. *World J Surg.* 1996;20: p 437-446.

4. Med, P.D.A.R. Neuropeptides and inflammation: the role of substance P. *Annu Rev Med.* 1989;40: p 341-352.

5. Hokfelt T, JO, Ljungdahl A, Lundberg JM, Schultzberg M. Nature. Peptidergic neurones. *Nature.* 1980;284: p 515-521.

6. Jessell TM, KD. *Principles of Neural Science.* Pain and analgesia., ed. S.J. Kandel ER, Jessell TM. New York, NY: Elsevier Science Inc; 1991:385-399.

7. Felten DL, FS, Bellinger DL. Noradrenergic sympathetic neural interactions with the immune system: structure and function. *Immunol Rev.* 1987;100: p 225-258.

8. Holzer P. Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. *Neuroscience.* 1988;24: p 739-768.

9. Hokfelt T, SH, Schmidt RF. *Neuropeptides, Nociception and Pain.* Weinheim, Germany: Chapman & Hall; 1994.

10. Kennis, P. Identification of the unmyelinated sensory nerves which evoke plasma extravasation in response to antidromic stimulation. *Neurosci Lett.* 1981; 25:p 137-141.

11. Holzer P. Peptidergic sensory neurons in the control of vascular functions: Mechanisms and significance in the cutaneous and splanchnic vascular beds. *Rev Physiol Biochem Pharmacol.* 1992;121: p 50-146.

12. Bayliss WM. On the origin from the spinal cord of the vasodialator fibres of the hind limb, and on the nature of these fibres. *J Physiol.* 1901; 26:p 173-209.

13. Jansco N, Jansco-Gabor A, Szolcsanyi J. Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. *Br J Pharmacol.* 1967;31: p 131-151.

14. Barnes PJ, Brown MJ, Dollery CT, et al. Histamine is release from from skin by SP but does not act as the final vasodialator orn the axon reflex. *Br J Pharmacol.* 1986;88:p 741-746.

15. Safieh-Garabedian B, Poole S, Allchone A, Winter J, Woolf CJ. Contribution of interlukin-1 beta to the inflammation-induced increase in NGF levels and inflammatory hyperalgesia. *Br J Pharmacol.* 1995; 115:1265-1275.

16. Hagermark O, Hokfelt T, Pernow B. Flare and itch induced by SP in human skin. *J Invest Dermato.* 1978;71: p 233-235.

17. Helme RD, Eglezos A, Hosking C S. Substance P induces chemotaxis of neutrophils in normal and capsaicin-treated rats. *Immunol Cell Biol.* 1987; 65: p 267-269.

18. Furness JB, Papka RE, Della NG, Costa M, Eskay RL. Substance P-like immunoreactivity in nerves associated with the vascular system of guinea pigs. *Neuroscience.* 1982;7:p 447-459.

19. Wallengren J, Hakanson R. Effects of SP, neurokinin A and calcitonin gene-related peptide in human skin and their involvement in sensory nerve-mediated responses. *Eur J Pharm.* 1987;143: p 267-273.

20. Ralevic V, Milner P, Hudlicka O, Kristek F, Burnstock G. Substance P is released from the endothelium of normal and capsaicin treated rat hind-limb vasculature in vivo, by increased flow. *Circ Res.* 1990; 66:p 1178-1183.

21. Dunnick CA, Gibran NS, Heimbach DM. Substance P has a role in neurogenic mediation of human burn wound healing. *J. Burn Care Rehabil.* 1996; 17: p. 390-396.

22. Blockers M, Benes P, Bork K. Persistant skin ulcers, mutilations and acro-osteolysis in hereditary sensory and autonomic neuropathy with phospholipid excretion. *J Am Acad Dermatol.* 1989;21: p 736-739.

23. Hagen NA, Stevens JC, Michet CJ. Trigeminal sensory neuropathy associated with connective tissue disease. *Neurology.* 1990;40:p 891-896.

24. Ziche M, Morbidelli L, Pacini M, et al. Substance P stimulates neovascularization in vivo and proliferation of cultured endothelial cells. *Microvasc Res.* 1990;40(264-278).

25. Namuri S, Fujita T. Stimulatory effects of SP and NGF on neurite outgrowth in embryonic chick dorsal root ganglia. *Neuropharmacology.* 1978;17(73-76).

26. Kahler CM, HM, Reinisch N, Wiedermann CJ. Interaction of SP with epidermal growth factor and fibroblast growth factor in cyclooxygenase-dependent proliferation of human skin fibroblasts. *J Cell Physiol.* 1996;166:p 601-608.

27. Ziche M, ML, Pacini M, Dolara P, Maggi CA. NK1-receptors mediate the proliferative response of human fibroblasts to tachykinins. *Br J Pharmacol.* 1990;100: p 11-14.

28. Zawacki BE, Jones RJ. Standard depth burns in the rat: The importance of the hair growth cycle. *Br J Plast Surg.* 1967;20:4 p 347-354

29. McMarthy BG, Hsieh ST, Stocks A, et al. Cutaneous innervation in sensory neuropathies: Evaluation by skin biopsy. *Nuerology* 1995;45 (10):1848-1855.

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††Dr McManus is assigned as a Senior Scientist, Laboratory Division.

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Oh Lord, I ask for the divine strength to meet the demands of my profession. Help me to be the finest medic, both technically and tactically. If I am called to the battlefield, give me the courage to conserve our fighting forces by providing medical care to all who are in need. If I am called to a mission of peace, give me the strength to lead by caring for those who need my assistance. Finally, Lord, help me to take care of my own spiritual, physical, and emotional needs. Teach me to trust in your presence and never-failing love.

Amen

*U.S. GPO: 2003-6718-S/20016

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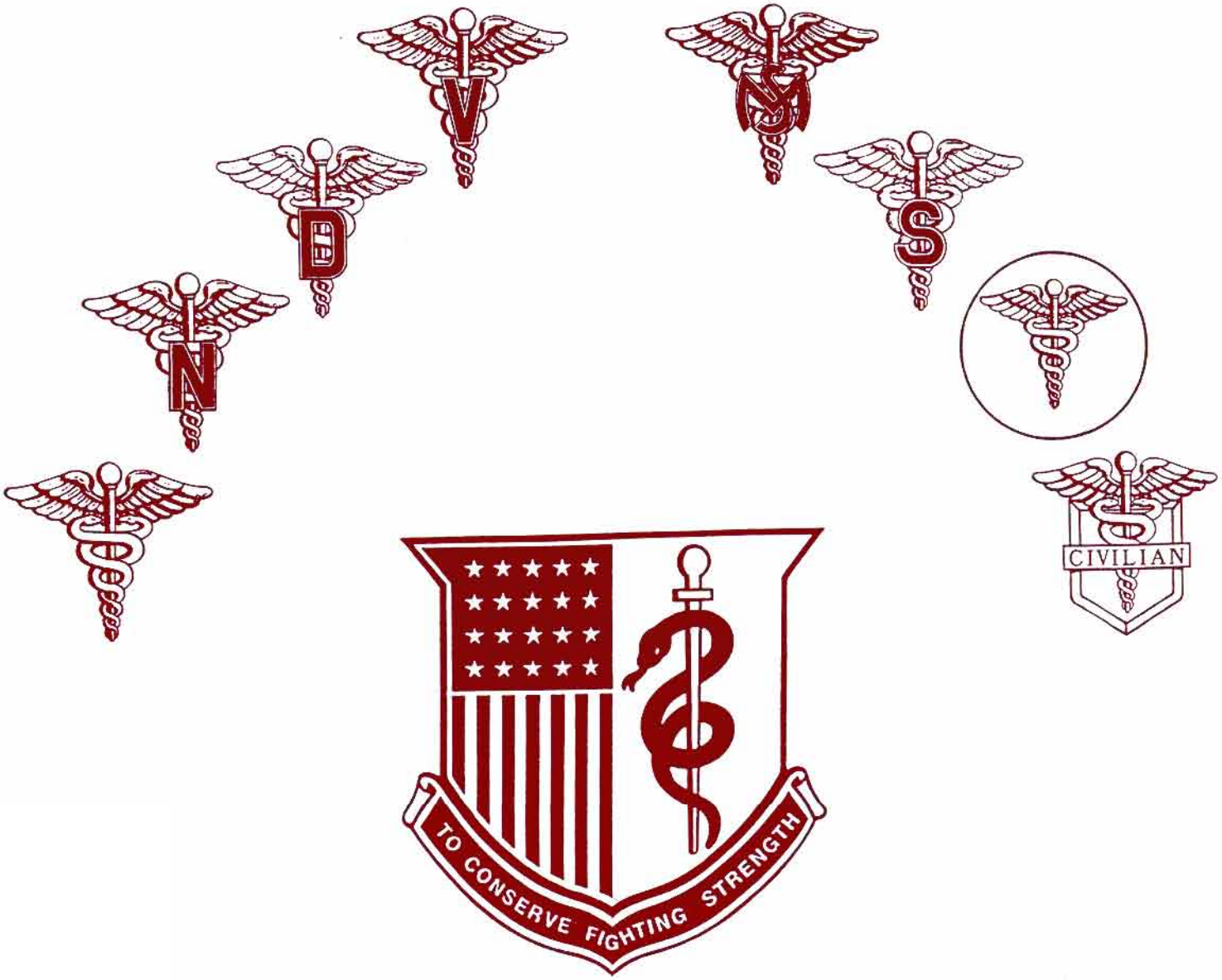

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